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(54) **Title:** RADIOACTIVELY LABELED 1,4-BENZOTHIAZEPINES AND METHODS OF SCREENING FOR COMPOUNDS THAT BIND RYANODINE RECEPTORS

(57) **Abstract:** The present invention provides certain radioactively labeled 1,4-benzothiazepine compounds that are useful in methods of screening for candidate compounds that bind a ryanodine receptor (RyR). The compounds are defined by structural formulae and the screening method includes the steps of incubating the RyR with a candidate compound in the presence or absence of a radioactively labeled compound so as to prepare a competitively bound-RyR composition containing RyR-bound candidate compound, RyR-bound radioactively labeled compound, or a combination thereof; separating the RyR-bound composition from un-bound radioactively labeled compound; measuring the radioactivity of the competitively RyR-bound composition; and determining whether the candidate compound binds the RyR based on the proportion of RyR-bound radioactively labeled compound in the presence and absence of the candidate compound.

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RADIOACTIVELY LABELED 1,4-BENZOTHIAZEPINES AND METHODS OF SCREENING FOR COMPOUNDS THAT BIND RYANODINE RECEPTORS

FIELD OF THE INVENTION

This invention relates to radioactively labeled compounds and the use thereof for identifying compounds useful in treating disorders and diseases associated with ryanodine receptors (RyRs). More particularly, the invention relates to radioactively labeled 1,4-benzothiazepines.

BACKGROUND OF THE INVENTION

The sarcoplasmic reticulum (SR) is a structure in cells that functions, among other things, as a specialized intracellular calcium (Ca^{2+}) store. RyRs are channels in the SR, which open and close to regulate the release of Ca^{2+} from the SR into the intracellular cytoplasm of the cell. Release of Ca^{2+} into the cytoplasm from the SR increases cytoplasmic Ca^{2+} concentration. Open probability of RyRs refers to the likelihood that a RyR channel is open at any given moment, and therefore capable of releasing Ca^{2+} into the cytoplasm from the SR.

There are three types of RyRs, all of which are highly-related Ca^{2+} channels: RyR1, RyR2, and RyR3. RyR1 is found predominantly in skeletal muscle as well as other tissues, RyR2 is found predominantly in the heart as well as other tissues, and RyR3 is found in the brain as well as other tissues. The RyR channels are formed by four RyR polypeptides in association with four FK506 binding proteins (FKBPs), specifically FKBP12 (calstabin1) and FKBP12.6 (calstabin2). Under physiological conditions, calstabin-1 binds selectively to RyR1 and RyR3, and calstabin-2 binds predominantly with RyR2. In the heart, calstabin binding helps stabilize the RyR2 channel by decreasing its open probability, minimizing calcium leak into the cytoplasm and thereby preventing aberrant activation during the resting phase of the cardiac cycle. Calstabin binding also facilitates coupled gating between neighboring RyR2 channels, which is thought to be important for promoting efficient excitation-contraction in muscle.

Calstabin binding to RyR can be regulated by covalent modifications to RyR. For example, calstabin-2 binding to RyR2 is regulated by protein kinase A (PKA)-mediated mediated phosphorylation of Ser2809 in RyR2. PKA phosphorylation of RyR2 decreases the

binding affinity of calstabin-2, causing calstabin-2 dissociation and increasing RyR2 open probability (Po) and its sensitivity to Ca^{2+}-dependent activation. More generally, PKA-phosphorylated RyR is an important part of the "fight or flight" response, by which the sympathetic nervous system (SNS), in response to stress or exercise, enhances myocardial contractility and increases cardiac output. In particular, the activated sympathetic nervous system produces catecholamines (noradrenalin and adrenalin), which stimulate a beta adrenergic signaling cascade that results in a transient increase in PKA-phosphorylation of RyR2 at S2809. By decreasing the binding affinity of RyR2 for calstabin-2, this modification alters the biophysical properties of the channel such that there is an increased SR calcium release for a given calcium trigger.

Ca^{2+} release from the SR in skeletal muscle and heart cells is a key physiological mechanism that controls muscle performance, because increased concentration of Ca^{2+} in the intracellular cytoplasm causes contraction of the muscle. Aberrations in intracellular calcium signaling are associated with a spectrum of diseases, including disorders of the heart, muscle, and brain. Disruptions in RyR function and its association with calstabin are implicated in at least some of these diseases.

Excitation-contraction (EC) coupling in skeletal muscles involves electrical depolarization of the plasma membrane in the transverse tubule (T-tubule), which activates voltage-gated L-type Ca^{2+} channels (LTCCs). LTCCs trigger Ca^{2+} release from the SR through physical interaction with RyR1. The resulting increase in cytoplasmic Ca^{2+} concentration induces actin-myosin interaction and muscle contraction. To enable relaxation, intracellular Ca^{2+} is pumped back into the SR via SR Ca^{2+}-ATPase pumps (SERCAs), which is regulated by phospholamban (PLB) depending on the muscle fiber type.

It has been shown that disease forms that result in sustained activation of the sympathetic nervous system and increased plasma catecholamine levels cause maladaptive activation of intracellular stress pathways resulting in destabilization of the RyR1 channel closed state and intracellular Ca^{2+} leak. SR Ca^{2+} leak via RyR1 channels has been found to deplete intracellular SR calcium stores, to increase compensatory energy consumption, and to result in significant acceleration of muscle fatigue. The stress-induced muscle defect permanently reduces isolated muscle and *in vivo* performance particularly in situations of increased demand.

It also has been shown that destabilization of RyR1 closed state occurs under pathologic conditions of increased sympathetic activation and involves depletion of the stabilizing calstabin1 channel subunit. Experiments have shown that PKA activation as an end effector of the sympathetic nervous systems increases PKA phosphorylation of RyR1 at Ser-2843 which decreases the binding affinity of calstabin1 to RyR1 and increases channel open probability.

In cardiac striated muscle, RyR2 is the major Ca^{2+}- release channel required for EC coupling and muscle contraction. During EC coupling, depolarization of the cardiac-muscle cell membrane during phase zero of the action potential activates voltage-gated Ca^{2+} channels. Ca^{2+} influx through the open voltage-gated channels in turn initiates Ca^{2+} release from the SR via RyR2. This process is known as Ca^{2+}-induced Ca^{2+} release. The RyR2-mediated, Ca^{2+}-induced Ca^{2+} release then activates the contractile proteins in the cardiac cell, resulting in cardiac muscle contraction.

Phosphorylation of cardiac RyR2 by PKA is an important part of the "fight or flight" response that increases cardiac EC coupling gain by augmenting the amount of Ca^{2+} released for a given trigger. This signaling pathway provides a mechanism by which activation of the sympathetic nervous system, in response to stress, results in increased cardiac output. PKA phosphorylation of RyR2 increases the open probability of the channel by dissociating calstabin2 from the channel complex. This, in turn, increases the sensitivity of RyR2 to Ca^{2+}-dependent activation.

Despite advances in treatment, heart failure remains an important cause of mortality in Western countries. An important hallmark of heart failure is reduced myocardial contractility. In heart failure, contractile abnormalities result, in part, from alterations in the signaling pathway that allows the cardiac action potential to trigger Ca^{2+} release *via* RyR2 channels and muscle contraction. In particular, in failing hearts, the amplitude of the whole-cell Ca^{2+} transient is decreased and the duration prolonged.

Cardiac arrhythmia, a common feature of heart failure, results in many of the deaths associated with the disease. Atrial fibrillation (AF) is the most common cardiac arrhythmia in humans, and represents a major cause of morbidity and mortality. Structural and electrical remodeling – including shortening of atrial refractoriness, loss of rate-related adaptation of refractoriness, and shortening of the wavelength of re-entrant wavelets – accompany

sustained tachycardia. This remodeling is likely important in the development, maintenance and progression of atrial fibrillation. Studies suggest that calcium handling plays a role in electrical remodeling in atrial fibrillation.

Approximately 50% of all patients with heart disease die from fatal cardiac arrhythmias. In some cases, a ventricular arrhythmia in the heart is rapidly fatal – a phenomenon referred to as "sudden cardiac death" (SCD). Fatal ventricular arrhythmias and SCD also occur in young, otherwise-healthy individuals who are not known to have structural heart disease. In fact, ventricular arrhythmia is the most common cause of sudden death in otherwise-healthy individuals.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disorder in individuals with structurally normal hearts. It is characterized by stress-induced ventricular tachycardia – a lethal arrhythmia that causes SCD. In subjects with CPVT, physical exertion and/or stress induce bidirectional and/or polymorphic ventricular tachycardias that lead to SCD even in the absence of detectable structural heart disease. CPVT is predominantly inherited in an autosomal-dominant fashion. Individuals with CPVT have ventricular arrhythmias when subjected to exercise, but do not develop arrhythmias at rest. Studies have identified mutations in the human RyR2 gene, on chromosome 1q42-q43, in individuals with CPVT.

Failing hearts (*e.g.*, in patients with heart failure and in animal models of heart failure) are characterized by a maladaptive response that includes chronic hyperadrenergic stimulation. In heart failure, chronic beta-adrenergic stimulation is associated with the activation of beta-adrenergic receptors in the heart, which, through coupling with G-proteins, activate adenylyl cyclase and thereby increase intracellular cAMP concentration. CAMP activates cAMP-dependent PKA, which has been shown to induce hyperphosphorylation of RyR2. Thus, chronic heart failure is a chronic hyperadrenergic state that results in several pathologic consequences, including PKA hyperphosphorylation of RyR2.

PKA hyperphosphorylation of RyR2 has been proposed as a factor contributing to depressed contractile function and arrhythmogenesis in heart failure. Consistent with this hypothesis, PKA hyperphosphorylation of RyR2 in failing hearts has been demonstrated, *in vivo*, both in animal models and in patients with heart failure undergoing cardiac transplantation.

In failing hearts, the hyperphosphorylation of RyR2 by PKA induces the dissociation of calstabin2 from the RyR2 channel. This causes marked changes in the biophysical properties of the RyR2 channel, including increased open probability due to an increased sensitivity to Ca^{2+}-dependent activation; destabilization of the channel, resulting in subconductance states; and impaired coupled gating of the channels, resulting in defective EC coupling and cardiac dysfunction. Thus, PKA-hyperphosphorylated RyR2 is very sensitive to low-level Ca^{2+} stimulation, and this manifests itself as a diastolic SR Ca^{2+} leak through PKA hyperphosphorylated RyR2 channel.

The maladaptive response to stress in heart failure results in depletion of calstabin2 from the channel macromolecular complex. This leads to a shift to the left in the sensitivity of RyR2 to Ca^{2+}-induced Ca^{2+} release, resulting in channels that are more active at low-to-moderate Ca^{2+} concentrations. Over time, the increased "leak" through RyR2 results in resetting of the SR Ca^{2+} content to a lower level, which in turn reduces EC coupling gain and contributes to impaired systolic contractility.

Additionally, a subpopulation of RyR2 that are particularly "leaky" can release SR Ca^{2+} during the resting phase of the cardiac cycle, diastole. This results in depolarizations of the cardiomyocyte membrane known as delayed after-depolarizations (DADs), which are known to trigger fatal ventricular cardiac arrhythmias.

In patients with CPVT mutations in their RyR2 and otherwise structurally-normal hearts, a similar phenomenon is at work. Specifically, it is known that exercise and stress induce the release of catecholamines that activate beta-adrenergic receptors in the heart. Activation of the beta-adrenergic receptors leads to PKA hyperphosphorylation of RyR2 channels. Evidence also suggests that PKA hyperphosphorylation of RyR2 resulting from beta-adrenergic-receptor activation renders mutated RyR2 channels more likely to open in the relaxation phase of the cardiac cycle, increasing the likelihood of arrhythmias.

Cardiac arrhythmias are known to be associated with diastolic SR Ca^{2+} leaks in patients with CPVT mutations in their RyR2 and otherwise structurally-normal hearts. In these cases, the most common mechanism for induction and maintenance of ventricular tachycardia is abnormal automaticity. One form of abnormal automaticity, known as triggered arrhythmia, is associated with aberrant release of SR Ca^{2+}, which initiates DADs. DADs are abnormal depolarizations in cardiomyocytes that occur after repolarization of a

cardiac action potential. The molecular basis for the abnormal SR Ca^{2+} release that results in DADs has not been fully elucidated. However, DADs are known to be blocked by ryanodine, providing evidence that RyR2 plays a key role in the pathogenesis of this aberrant Ca^{2+} release.

U.S. Patent No. 6,489,125 discusses JTV-519 (4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride; also known as k201 or ICP-Calstan 100), a 1,4-benzothiazepine, as a new modulator of RyR calcium-ion channels. U.S. Published Patent Application No. 2005/0187386 discusses RyR2 as a target for treating and preventing heart failure and cardiac arrhythmias, including atrial fibrillation and cardiac arrhythmias that cause exercise-induced SCD. RyR2 channels with 7 different CPVT mutations (*e.g.*, S2246L, R2474S, N4104K, R4497C, P2328S, Q4201R, V4653F) were found to have functional defects that resulted in channels that became leaky (*i.e.*, a calcium leak) when stimulated during exercise. The mechanism for the VT in CPVT has been demonstrated to be the same as the mechanism for VT in heart failure.

It has been shown that exercise-induced arrhythmias and sudden death (in patients with CPVT) result from a reduced affinity of calstabin2 for RyR2. Additionally, it has been demonstrated that exercise activates RyR2 as a result of phosphorylation by 3', 5'-cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA). Mutant RyR2 channels, which had normal function in planar lipid bilayers under basal conditions, were more sensitive to activation by PKA phosphorylation – exhibiting increased activity (open probability) and prolonged open states, as compared with wild-type channels. In addition, PKA-phosphorylated mutant RyR2 channels were resistant to inhibition by Mg^{2+}, a physiological inhibitor of the channel, and showed reduced binding to calstabin2, which stabilizes the channel in the closed state. These findings indicate that, during exercise, when RyR2 are PKA-phosphorylated, the mutant CPVT channels are more likely to open in the relaxation phase of the cardiac cycle, increasing the likelihood of arrhythmias triggered by SR Ca^{2+} leak.

U.S. Published Patent Application No. 2003/0134331 discusses a method for regulating contraction of a subject's heart by administering a compound that regulates PKA phosphorylation of an RyR2 and specifically decreases PKA phosphorylation. U.S. Published Patent Application No. 2004/0048780 also discusses a method for treating and

preventing atrial tachyarrhythmia and exercise- and stress-induced arrhythmias by administration of an agent which inhibits PKA phosphorylation of RyR2.

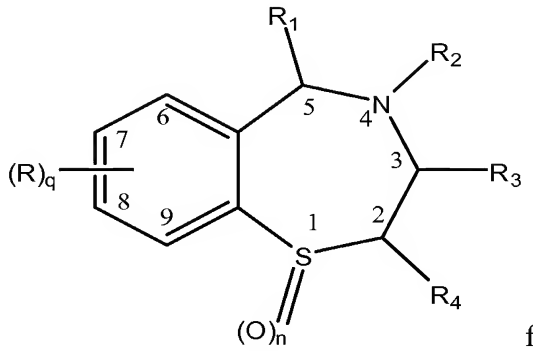
Additionally, U.S. Published Patent Application Nos. 2006/0194767 and 20070173482 discuss a series of 1,4-benzothiazepines, as well as methods of preparing the same, as compounds capable of treating disorders and diseases associated with RyRs that regulate calcium channel functioning in cells.

In view of the foregoing, however, there is a need to identify new compounds, as well as compounds with high efficacy, for treating disorders and diseases associated with RyRs that regulate calcium channel functioning in cells.

SUMMARY OF THE INVENTION

The present invention now provides novel radioactively labeled compounds and their use in screening methods for identifying compounds having an affinity for binding to a ryanodine receptor (RyR) or for determining the affinity of an active compound for an RyR.

The radioactively labeled compounds are certain 1,4-benzothiazepines defined by formula I:



formula I

wherein:

n is selected from the group consisting of 0, 1 and 2;

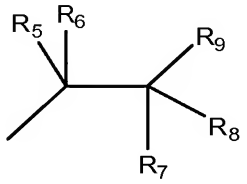
q is selected from the group consisting of 0, 1, 2, 3, and 4;

each R is independently selected from the group consisting of halogen, R_4,

$-OR_4$; $-N(R_4)_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3R_4$, $-S(=O)_2R_4$, $-S(=O)R_4$, $OS(=O)_2CF_3$, acyl, -O-acyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, alkylaryl, heterocyclylalkyl, alkynyl, (hetero-)arylthio, and (hetero-) arylamino;

R_1 is selected from the group consisting of oxo (=O) and R_4;

R_2 is selected from the group consisting of R_4 and a group of the formula:



R_3 is selected from the group consisting of oxo (=O), R_4, $-CO_2R_4$, $-C(=O)N(R_4)_2$, acyl, and -O-acyl;

each R_4 is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; and

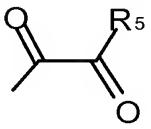
R_5, R_6, R_7, R_8 and R_9 are each independently selected from the group consisting of halogen, R_4, $-OR_4$, $-N(R_4)_2$, $-N(R_4)_2N(R_4)_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3R_4$, $-S(=O)_2R_4$, $-S(=O)R_4$, $-OS(=O)_2CF_3$, acyl, -O-acyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, heterocyclylalkyl, alkynyl, (hetero-)arylthio, and (hetero-) arylamino; or R_5 and R_6, taken together, may form an oxo (=O), thio (=S) or imino ($=NR_4$); or R_7 and R_8, taken together, may form an oxo (=O), thio (=S) or imino ($=NR_4$);

wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)arylthio, and (hetero-)arylamino is unsubstituted or substituted with one or more groups selected from the group consisting of halogen, CF_3, OCF_3, cyano, nitro, N_3, oxo, alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, aryl, alkylaryl, heteroaryl, OR_a, SR_a, $S(=O)R_e$, $S(=O)_2R_e$, $P(=O)_2R_e$, $S(=O)_2OR_a$, $P(=O)_2OR_a$, NR_bR_c, $NR_bS(=O)_2R_e$, $NR_bP(=O)_2R_e$, $S(=O)_2NR_bR_c$, $P(=O)_2NR_bR_c$, $C(=O)OR_a$, $C(=O)R_a$, $C(=O)NR_bR_c$, $OC(=O)R_a$, $OC(=O)NR_bR_c$, $NR_bC(=O)OR_a$, $NR_dC(=O)NR_bR_c$, $NR_dS(=O)_2NR_bR_c$, $NR_dP(=O)_2NR_bR_c$, $NR_bC(=O)R_a$, or $NR_bP(=O)_2R_e$, wherein R_a, R_b, R_c, R_d and R_e are each

independently R_4; or said R_b and R_c together with the N to which they are bonded optionally form a heterocyclyl or heteroaryl;

wherein at least one atom in the compound of formula I is radioactively labeled. The present invention further includes the enantiomers, diastereomers, tautomers, salts, hydrates, solvates, complexes, and prodrugs of the compounds defined by formula I.

In a preferred embodiment of the present invention, the radioactively labeled 1,4-benzothiazepines are selected from radioactively labeled compounds of formula I wherein formula I comprises at least one radioactive atom and R_2 is selected from the group consisting of alkyl, alkenyl, alkynyl and



Other embodiments of the present invention relate to a method of screening candidate compounds that bind a ryanodine receptor (RyR) comprising the steps of: (i) incubating the RyR with a candidate compound in the presence or absence of a radioactively labeled compound so as to prepare a competitively bound-RyR composition containing RyR-bound candidate compound, RyR-bound radioactively labeled compound, or a combination thereof; (ii) separating the RyR-bound composition from un-bound radioactively labeled compound; (iii) measuring the radioactivity of the competitively RyR-bound composition; and (iv)determining whether the candidate compound binds the RyR based on the proportion of RyR-bound radioactively labeled compound in the presence and absence of the candidate compound. The RyR in those embodiments is selected from the group consisting of RyR1, RyR2 and RyR3.

The invention further relates to a method of treating a disease or disorder associated with an RyR by administering to a patient in need thereof a therapeutically active compound having the ability to bind the RyR identified by the methods disclosed above.

The invention also provides for methods of preparing certain radiolabeled compounds according to formulae I, II and III, including, but not limited to, compounds 1, 1a, 1b, 2, 2a and 3, as defined herein.

In another embodiment of the present invention, the invention provides for a method of preparing a radiolabeled compound according to formula I which comprises reacting an intermediate compound with a radioactive moiety to form a radiolabeled compound.

In yet another embodiment of the present invention, the invention provides for a method of measuring tissue distribution of a radioactively labeled compound, which method comprises administering the radioactively labeled compound to a subject and measuring the amount of the radioactively labeled compound in the subject.

In yet another embodiment of the present invention, the invention provides for a method of measuring the tissue distribution of a radioactively labeled compound, which method comprises isolating a tissue from a subject, contacting the tissue with the radioactively labeled compound and measuring the amount of the radioactively labeled compound in the tissue.

In a further embodiment, the invention provides for the use of a radioactively labeled compound of the present invention in the preparation of a pharmaceutical composition that can be administered for determining binding to ryanodine receptors or for measuring tissue distribution in a subject.

Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

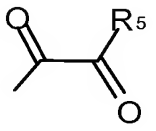
The present invention now provides novel radioactively labeled compounds and their use in screening methods for identifying compounds having an affinity for binding to an RyR or for determining the affinity of an active compound for an RyR. The radioactively labeled compounds in the present invention are certain 1,4-benzothiazepines wherein the compound comprises at least one radioactive atom.

Compounds

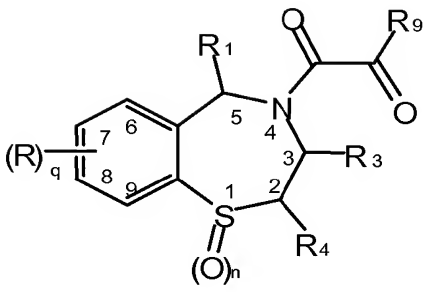
In certain embodiments of the present invention, the invention provides radioactively labeled compounds. In some embodiments of the present invention, the radioactively labeled compounds are compounds with a known affinity for an RyR. In other embodiments of the present invention, the invention provides radioactively labeled 1,4-benzothiazepines.

The radioactively labeled compounds of the present invention are labeled with any know radioactive atom. In some embodiments of the present invention, radioactive atoms are selected from, by way of non-limiting example, tritium (^{3}H or T, used herein interchangeably), carbon-14 (^{14}C), radioactive phosphorus isotopes, such as phosphorus-32 (^{32}P) and phosphorus-33 (^{33}P), sulfur-35 (^{35}S), and radioactive halogens, which include, but are not limited to, chlorine-36 (^{36}Cl), iodine-123 (^{123}I), iodine-125 (^{125}I), iodine-126 (^{126}I), iodine-129 (^{129}I) and iodine-131 (^{131}I). In some embodiments of the present invention, the radioactively labeled compound comprises a single radioactive atom. In some embodiments of the present invention, the radioactively labeled compound comprises two or more radioactive atoms. In some embodiments the radioactive atom(s) will be in the backbone or side-chains of the radiolabel compound, or both.

In a preferred embodiment of the present invention, the radioactively labeled 1,4-benzothiazepines are selected from radioactively labeled compounds of formula I wherein formula I comprises at least one radioactive atom and R_2 is selected from the group consisting of alkyl, alkenyl, alkynyl and



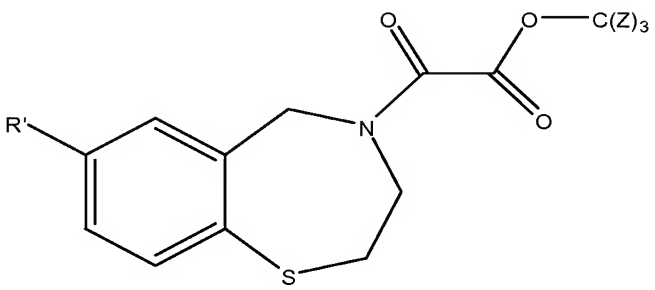
Thus, one preferred embodiment of the present invention includes the radioactively labeled 1,4-benzothiazepines selected from radioactively labeled compounds of formula II, wherein formula II comprises at least one radioactive atom and is represented by the formula:



formula II

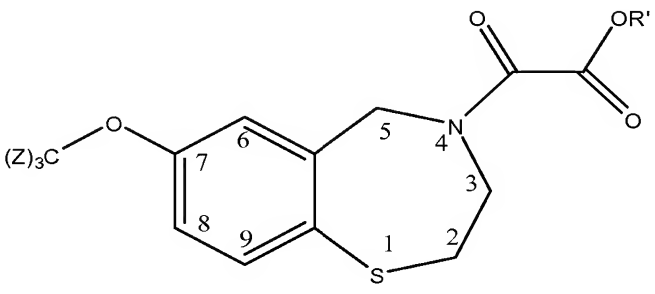
wherein each of n, q, R, R_1, R_3, R_4 and R_9 are as defined previously. All enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, and complexes of the radioactively labeled compounds of formula II are contemplated by the present invention.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



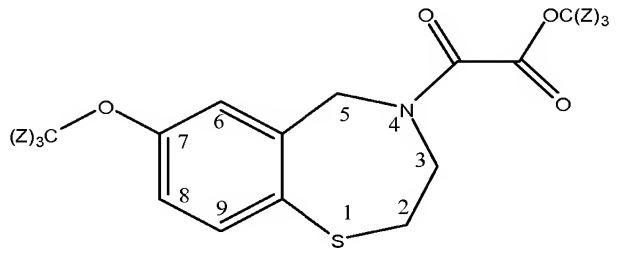
wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F. In one embodiment of this formula, R' is H and each Z, independently of the other, is hydrogen or ^{3}H, with at least one Z being ^{3}H. In another embodiment of this formula, R' is methoxy and each Z, independently of the other, is hydrogen or ^{3}H, with at least one Z being ^{3}H.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



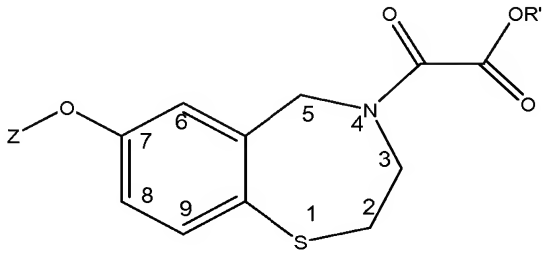
wherein R' is hydrogen or methyl and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F. In one embodiment of this formula, R' is H and each Z, independently of the other, is hydrogen or ^{3}H, with at least one Z being ^{3}H. In another embodiment of this formula, R' is methoxy and each Z, independently of the other, is hydrogen or ^{3}H, with at least one Z being ^{3}H.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



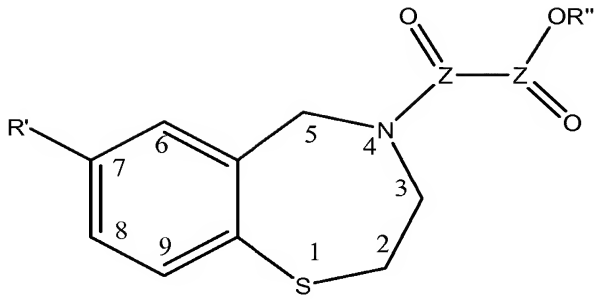
wherein each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



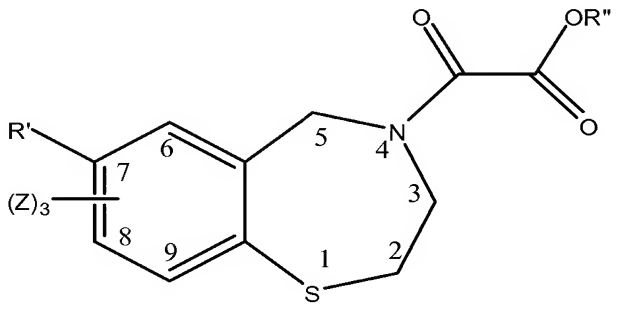
wherein R' is hydrogen or methyl and Z is $^{11}CH_3$ or $^{14}CH_3$.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



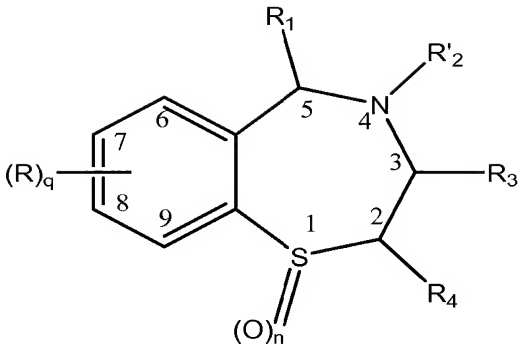
wherein R' hydrogen or methoxy, and R'' is hydrogen or methyl, and each Z, independently of the other, is C, ^{11}C or ^{14}C, with at least one Z being ^{11}C or ^{14}C. In one embodiment of this formula, wherein R' is methoxy and R'' is methyl. In another embodiment of this formula, wherein R' is methoxy and R'' is hydrogen.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by the formula:



wherein R' is hydrogen or methoxy, and R'' is hydrogen or methyl, and each Z, independently of the other, hydrogen, ^{3}H, ^{18}F or ^{125}I, with at lease one Z being ^{3}H, ^{18}F or ^{125}I. In one embodiment of this formula, R' is methoxy, R'' is methyl and Z is ^{3}H or ^{125}I. In another embodiment of this formula, R' is methoxy, R'' is hydrogen and Z is ^{3}H or ^{125}I.

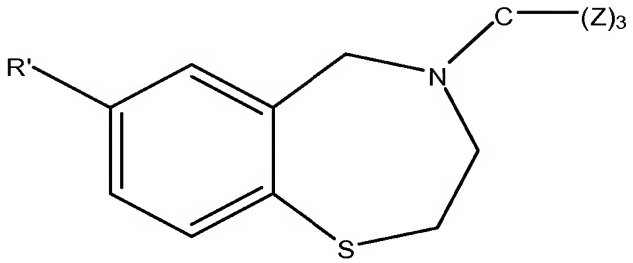
In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by formula III:



formula III

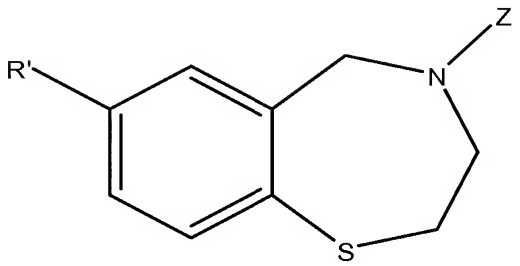
wherein R'_2 is alkyl, alkenyl or alkynyl, and wherein each of n, q, R, R_1, R_3 and R_4 are as defined previously. R'_2 is preferably an alkyl group.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by formula:



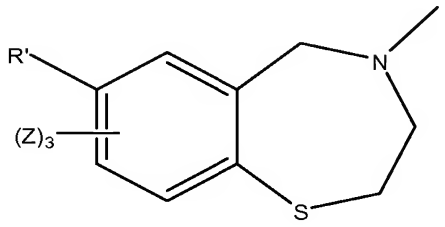
wherein R' is hydrogen or methoxy, and each Z, independently of the other, is hydrogen, ^3H or ^{18}F, with at lease one Z being ^3H or ^{18}F. In one embodiment of this formula, R' is H and each Z, independently of the other, is hydrogen or ^3H, with at lease one Z being ^3H. In another embodiment of this formula, R' is methoxy and each Z, independently of the other, is hydrogen or ^3H, with at lease one Z being ^3H.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by formula:



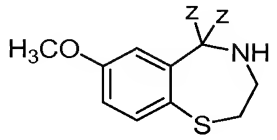
wherein Z is $^{11}CH_3$ or $^{14}CH_3$, and R' is hydrogen or methoxy. In one embodiment of this formula, R' is hydrogen. In another embodiment of this formula, wherein R' is methoxy.

In some preferred embodiments of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by formula:



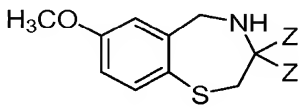
wherein R' is hydrogen or methoxy, and each Z, independently of the other, is hydrogen, ^{3}H, ^{18}F, or ^{125}I, with at lease one Z being ^{3}H, ^{18}F or ^{125}I. In one embodiment of this formula, R' is methoxy and Z is ^{3}H or ^{125}I. In another embodiment of this formula, R' is hydrogen and Z is ^{3}H or ^{125}I.

In another preferred embodiment of the present invention, the radioactively labeled 1,4- benzothiazepines are represented by formula:



wherein each Z is H or ^{3}H, wherein at least one Z is ^{3}H.

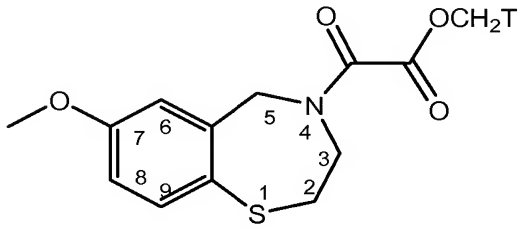
In other preferred embodiments of the present invention, the radioactively labeled 1,4-benzothiazepines are represented by formula:



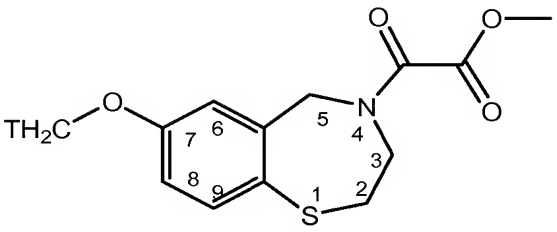
wherein each Z is H or ^{3}H, wherein at least one Z is ^{3}H.

In specific embodiments of the present invention, the radioactively labeled 1,4-benzothiazepine compound is **$^{3}H_1$-Compound 1-a**. In another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **$^{3}H_1$-Compound 1-**

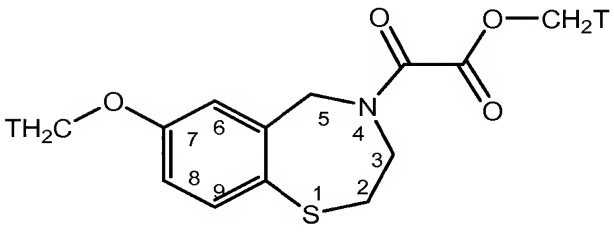
b. In still another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **$^{3}H_{2}$-Compound 1**. In yet another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **^{3}H-Compound 2**. In yet another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **^{3}H-Compound 2a**. In yet another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **^{3}H-Compound 3**. In yet another specific embodiment of the present invention, the radioactively labeled benzothiazepine compound is **^{14}C-Compound 3**.



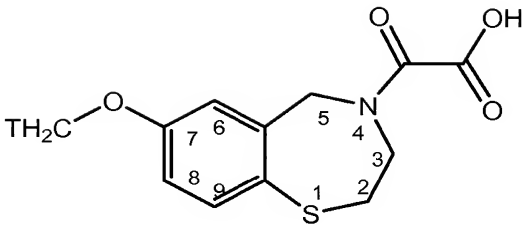
$^{3}H_{1}$-Compound 1-a



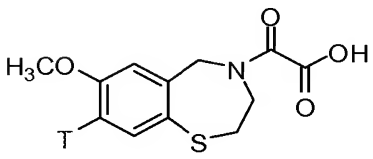
$^{3}H_{1}$-Compound 1-b



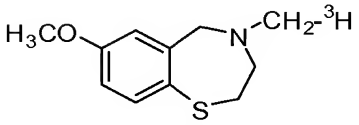
$^{3}H_{2}$-Compound 1



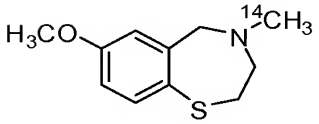
^{3}H-Compound 2



^{3}H-Compound 2a



^{3}H- Compound 3

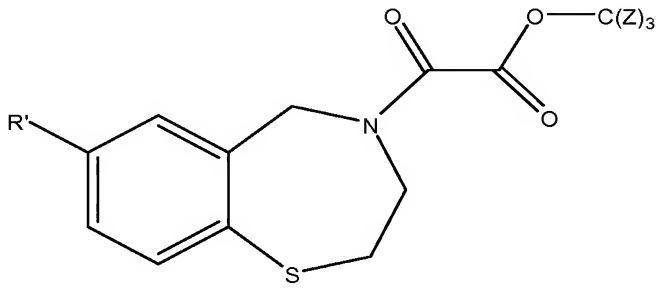


^{14}C- Compound 3

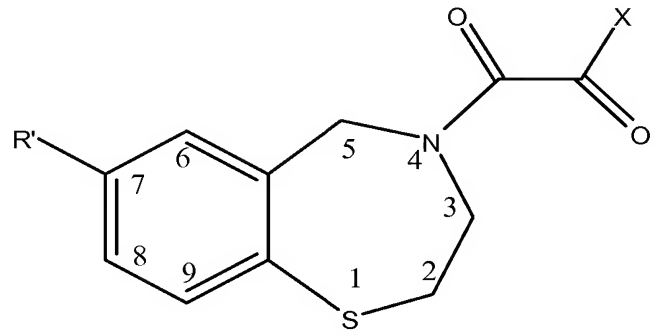
It will be apparent to a person of skill in the art, that radiolabelled reagents such as tritiated methanol or tritiated methyl iodide, may include any or all of the hydrogens substituted by ^{3}H (tritium or T). By way of illustration and not by limitation, tritiated methyl iodide can include TCH_2-I, T_2CH-I or T_3C-I, and may contain a mixture containing any or all of these molecules. It will further be apparent to a person of skill in the art that more than one hydrogen on the methyl at the 7-position and/or the methyl on the oxalic acid moiety can be substituted by tritium (^{3}H or T). Thus, the present invention contemplates any one of the aforementioned compounds wherein one or more CH_2T groups is replaced with CT_2H or CT_3 or any combination thereof.

In a method according to the present invention, a radiolabeled compound is prepared according to formula I by preparing an intermediate compound that contains a -C=OX moiety wherein X is a leaving group, for example a halogen or a sulfonyl, and treating the intermediate compound with ^{3}H-ROH under conditions sufficient to substitute a ^{3}H-RO moiety for the leaving group of the intermediate compound to form a radiolabeled compound.

In another embodiment, a radiolabeled compound of the following formula:

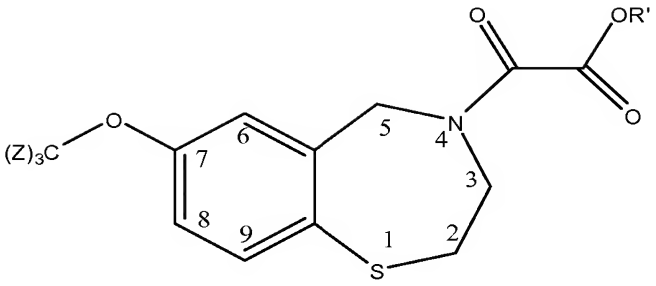


wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F, is prepared by preparing an intermediate of the formula:

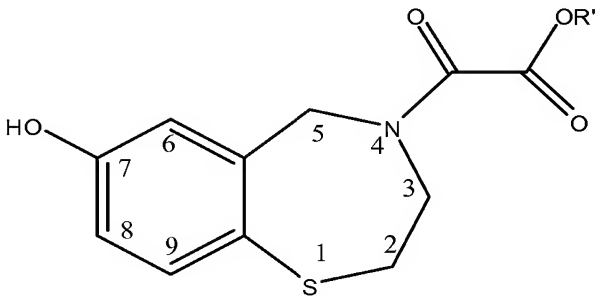


wherein X is a leaving group, and treating the intermediate compound with $(Z)_3COH$ under conditions sufficient to substitute a $(Z)_3CO$ moiety for the X moiety of the intermediate compound to form a radiolabeled compound.

In another embodiment, a radiolabeled compound of the following formula:

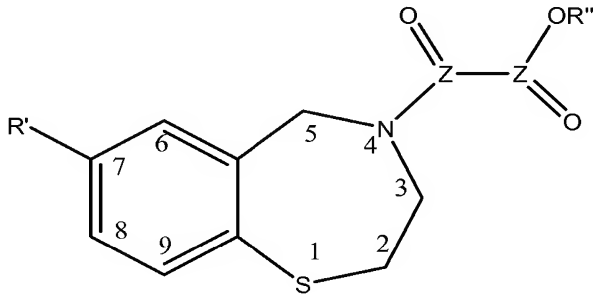


wherein R' is hydrogen or methyl and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F, is prepared by preparing an intermediate of the formula:

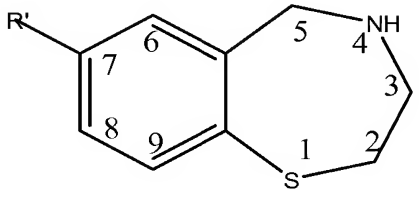


and treating the intermediate compound with $(Z)_3CX$ wherein X is a leaving group under conditions sufficient to convert the hydroxyl moiety of the intermediate compound into a $(Z)_3CO$ moiety to form a radiolabeled compound.

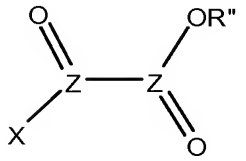
In another embodiment, a radiolabeled compound of the following formula:



wherein R' hydrogen or methoxy, and R'' is hydrogen or methyl, and each Z, independently of the other, is C, ^{11}C or ^{14}C, with at least one Z being ^{11}C or ^{14}C, is prepared by preparing an intermediate of the formula:

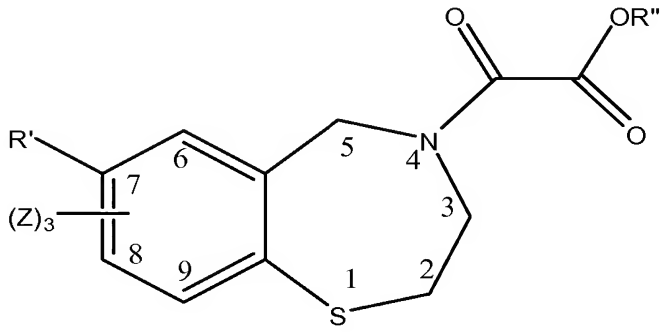


and reacting the intermediate compound with a group of the formula:

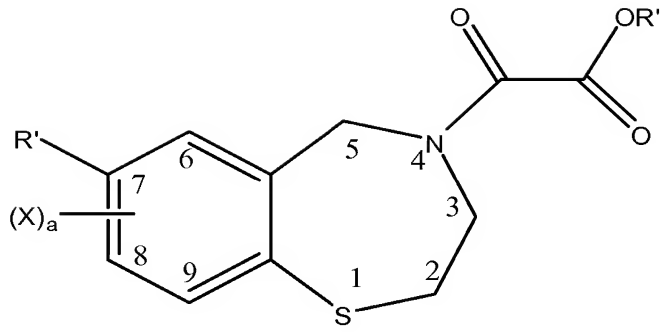


wherein X is a leaving group under conditions sufficient to form a radiolabeled compound.

In another embodiment, a radiolabeled compound of the following formula:



wherein R' is hydrogen or methoxy, and R'' is hydrogen or methyl, and each Z, independently of the other, is hydrogen, ^{3}H, ^{18}F or ^{125}I, with at least one Z being ^{3}H, ^{18}F or ^{125}I, is prepared by preparing an intermediate compound having the formula



wherein X is a leaving group and a is an integer selected from 1, 2 and 3; and substituting at least one X moiety with Z so as to form a radiolabeled compound.

Leaving group means the group with the meaning conventionally associated with it in synthetic organic chemistry, i.e., an atom or group displaceable under substitution reaction conditions. Examples of leaving groups include, but are not limited to, halogen (e.g., F, Cl, Br, I), alkane- or arylenesulfonyloxy, such as methanesulfonyloxy, ethanesulfonyloxy, thiomethyl, benzenesulfonyloxy, tosyloxy, and thienyloxy, dihalophosphinoyloxy, optionally substituted benzyloxy, isopropyloxy, acyloxy, and the like. Other examples of leaving groups include typical acyl-activating groups such as, without limitation, N-hydroxy succinimide, N-hydroxy benzotriazole etc. Also, the leaving group include acyl activating groups such as DCC, EDC, carbonyl diimidazole and the like, such that a carboxylic acid C(=O)OH is activated *in situ* to C(=O)-X which is then converted to the radioactively labeled compound without isolating the intermediates.

In another embodiment of the present invention, the invention provides for a method of preparing a radiolabeled compound according to formula I which comprises reacting an intermediate compound with radioactive moiety to form a radioactively labeled compound.

Definitions

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the content clearly dictates otherwise. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

The term "alkyl" as used herein refers to a linear or branched, saturated hydrocarbon having from 1 to 6 carbon atoms. Representative alkyl groups include, but are not limited to,

methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, and neohexyl.

The term "alkenyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon double bond. In one embodiment, the alkenyl has one or two double bonds. In some embodiments of the present invention, the alkenyl moiety exists in the E or Z conformation and the compounds of the present invention include both conformations.

The term "alkynyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon triple bond.

The term "aryl" as used herein refers to an aromatic group containing 1 to 3 aromatic rings, either fused or linked.

The term "cyclic group" as used herein includes a cycloalkyl group and a heterocyclic group.

The term "cycloalkyl group" as used herein refers to a three- to seven-membered saturated or partially unsaturated carbon ring. Any suitable ring position of the cycloalkyl group is covalently linked to the defined chemical structure. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

The term "heterocyclic group" or “heterocyclic” or “heterocyclyl” or “heterocyclo” as used herein refers to fully saturated, or partially or fully unsaturated, including aromatic (i.e., “heteroaryl”) cyclic groups (for example, 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom has 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms is, optionally, oxidized and the nitrogen heteroatoms are, optionally, quaternized. The heterocyclic group is attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. Exemplary heterocyclic groups include, but are not limited to, azepanyl, azetidinyl, aziridinyl, dioxolanyl, furanyl, furazanyl, homo piperazinyl, imidazolidinyl, imidazolinyl,

isothiazolyl, isoxazolyl, morpholinyl, oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, piperazinyl, piperidinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazolyl, pyridoimidazolyl, pyridothiazolyl, pyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, quinuclidinyl, tetrahydrofuranyl, thiadiazinyl, thiadiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiomorpholinyl, thiophenyl, triazinyl, and triazolyl. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, benzofurazanyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), triazinylazepinyl, tetrahydroquinolinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, acridinyl, phenanthridinyl, xanthenyl and the like.

The term "phenyl" as used herein refers to a substituted or unsubstituted phenyl group.

The aforementioned terms “alkyl,” “alkenyl," “alkynyl,” “aryl,” “phenyl,” “cyclic group,” “cycloalkyl,” “heterocyclyl,” “heterocyclo,” and “heterocycle” is further, optionally, substituted with one or more substituents. Exemplary substituents include but are not limited to one or more of the following groups: hydrogen, halogen, CF_3, OCF_3, cyano, nitro, N_3, oxo, cycloalkyl, alkenyl, alkynyl, heterocycle, aryl, alkylaryl, heteroaryl, OR_a, SR_a, $S(=O)R_e$, $S(=O)_2R_e$, $P(=O)_2R_e$, $S(=O)_2OR_a$, $P(=O)_2OR_a$, NR_bR_c, $NR_bS(=O)_2R_e$, $NR_bP(=O)_2R_e$, $S(=O)_2NR_bR_c$, $P(=O)_2NR_bR_c$, $C(=O)OR_a$, $C(=O)R_a$, $C(=O)NR_bR_c$, $OC(=O)R_a$, $OC(=O)NR_bR_c$, $NR_bC(=O)OR_a$, $NR_dC(=O)NR_bR_c$, $NR_dS(=O)_2NR_bR_c$, $NR_dP(=O)_2NR_bR_c$, $NR_bC(=O)R_a$, or $NR_bP(=O)_2R_e$, wherein R_a is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl; R_b, R_c and R_d are independently hydrogen, alkyl, cycloalkyl, alkylaryl, heteroaryl, heterocycle, aryl, or said R_b and R_c together with the N to which they are bonded optionally form a heterocycle; and R_e is alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl. In the aforementioned exemplary substitutents, groups such as alkyl, cycloalkyl, alkenyl, alkynyl, cycloalkenyl, alkylaryl, heteroaryl, heterocycle and aryl can themselves be optionally substituted.

The term "prodrug" as employed herein denotes a compound that, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield compounds of the present invention.

All stereoisomers of the radioactively labeled compounds of the present invention (for example, those which exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. In some embodiments of the present invention, individual stereoisomers of the compounds of the invention are, for example, substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or are admixed, for example, as racemates or with all other, or other selected, stereoisomers. In certain embodiments of the present invention, the chiral centers of the present invention have the S or R configuration as defined by the IUPAC 1974 Recommendations. In another embodiment of the present invention, the racemic forms are contemplated and are resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. In yet another embodiment of the present invention, the individual optical isomers are obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

All configurational isomers of the radioactively labeled compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of radioactively labeled compounds of the present invention embraces both cis (*Z*) and trans (*E*) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings.

Throughout the specifications, groups and substituents thereof are chosen to provide stable moieties and compounds.

As used herein, the terms "measuring" and "determining" are intended to include processes of both qualitative and quantitative measurements and determinations, respectively.

The present invention provides radioactively labeled compounds that are capable of being utilized in methods of screening candidate compound for active compounds that have an ability to bind or an affinity for an RyR, and a process for determining the affinity of an

active compound for an RyR. Compounds with an ability to bind or an affinity for RyRs are capable of treating disorders and diseases associated with RyRs that regulate calcium channel functioning in cells. "Disorders and diseases associated with RyRs" means disorders and diseases that can be treated by modulating RyRs that regulate calcium channel functioning in cells. "Disorders and diseases associated with RyRs" include, without limitation, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss.

Screening Methods

In some embodiments of the present invention, the invention provides an efficient method for screening (an assay) for compounds that have an ability to bind, or have an affinity for, an RyR. The methods are amenable to automated, cost-effective high throughput drug screening and have immediate application in a broad range of pharmaceutical and biotechnology drug development programs.

In one embodiment of the present invention, the method of screening for candidate compounds that bind a ryanodine receptor (RyR) comprising the steps of:

(i) incubating the RyR with a radioactively labeled compound in the presence or absence of a non-radioactive candidate compound so as to prepare a competitively bound-RyR composition containing RyR-bound candidate compound, RyR-bound radioactively labeled compound, or a combination thereof;

(ii) separating the RyR-bound composition from un-bound radioactively labeled compound;

(iii) measuring the radioactivity of the competitively RyR-bound composition; and

(iv) determining whether the candidate compound binds the RyR based on the proportion of RyR-bound radioactively labeled compound in the presence and absence of the candidate compound.

This invention envisions a method of screening for compounds that have an ability to bind (i.e. that have an affinity for) an RyR by utilizing radioactively labeled compounds having a known affinity for an RyR wherein the affinity for the RyR of a radioactively labeled compound is known for the radioactively labeled compound itself, the non-radioactively labeled analog of the radioactively labeled compound, or both. In those embodiments wherein the affinity for an RyR is known for the non-radioactively labeled analog rather than the radioactively labeled compound itself, it is assumed that any isotopic variations between the affinity for the RyR of the radioactively labeled compound and the non-radioactively labeled analog thereof is negligible for the purposes of screening for compounds that have an ability to bind an RyR according to the methods of the present invention.

In some embodiments of the present invention, the radioactively labeled compound having a known affinity for the RyR is known to, qualitatively, bind the RyR. In other embodiments of the present invention, the affinity of the radioactively labeled compound is known, quantitatively, for the RyR. In some embodiments of the present invention, a radioactively labeled compound having a known affinity for the RyR is a compound that completely binds the RyR under the incubation conditions of step (i) when the candidate compound is not present (non-competitive incubation),

In some embodiments of the present invention, a radioactively labeled compound having a known affinity for an RyR completely binds the RyR when it binds at least 99% of the RyR under the incubation conditions of step (i) when the candidate compound is not present. In other embodiments of the present invention, a radioactively labeled compound having a known affinity for an RyR completely binds the RyR when at least 98% of the RyR is bound to the radioactively labeled compound under the incubation conditions of step (i) when the candidate compound is not present. In still other embodiments of the present invention, a radioactively labeled compound having a known affinity for an RyR completely

binds the RyR when at least 95% of the RyR is bound to the radioactively labeled compound under the incubation conditions of step (i) when the candidate compound is not present. In yet other embodiments of the present invention, a radioactively labeled compound having a known affinity for an RyR completely binds the RyR when at least 90% of the RyR is bound to the radioactively labeled compound under the incubation conditions of step (i) when the candidate compound is not present. Compounds identified as having an ability to bind, or having an affinity for, an RyR according to one embodiment of the present invention are those compounds capable of binding to the RyR when competitively incubated with a radioactively labeled compound having a known affinity for the RyR. In some embodiments of the present invention, the compounds identified as having an ability to bind, or having an affinity for, an RyR, are those compounds that bind at least 1% of the RyR competitively incubated in step (i). In other embodiments of the present invention, the compounds identified as having an ability to bind, or having an affinity for, an RyR, are those compounds that bind at least 5% of the RyR competitively incubated in step (i). In another embodiment of the present invention, the compounds identified as having an ability to bind, or having an affinity for, an RyR, are those compounds that bind at least 10% of the RyR competitively incubated in step (i). In yet another embodiment of the present invention, the compounds identified as having an ability to bind, or having an affinity for, an RyR, are those compounds that bind at least 50% of the RyR competitively incubated in step (i). In still another embodiments of the present invention, the compounds identified as having an ability to bind, or having an affinity for, an RyR, are those compounds that bind at least 95% of the RyR competitively incubated in step (i).

A candidate compound is any compound for which a determination of affinity, or lack thereof, for RyR is desired. The method of the present invention is utilized for screening for compounds that have an affinity for any of the RyRs, including RyR1, RyR2 and RyR3.

In some embodiments of the present invention, step (i), the incubation of the RyR with a mixture of a candidate compound and a radioactively labeled compound having a known affinity for the RyR, is carried out at any temperature and for any length of time sufficient for a radioactively labeled compound to bind to the RyR. In some embodiments of the present invention, the incubation temperature in step (i) is room temperature. In other embodiments of the present invention, the incubation temperature in step (i) is at least 20°C. In one embodiment of the present invention, the incubation time in step (i) is at least about 30

minutes, e.g., at least about 1hr or at least about 2hr. In other embodiments of the present invention, the incubation time in step (i) is at least 1 hour. In another embodiment of the present invention, the incubation time in step (i) is about 1 hour.

In one embodiment of the present invention, step (ii) of the screening method comprises separating the RyR-bound composition from un-bound radioactively labeled compound, i.e. the radioactively labeled compound that is not bound to the RyR utilized in step (i). In some embodiments of the present invention, step (ii) comprises separating the RyR-bound radioactively labeled compound from un-bound radioactively labeled compound by any process of separation known in the art including, but not limited to, filtration, decanting, washing, extraction, precipitation and combinations thereof. In some embodiments of the present invention, at least 99% of the un-bound radioactively labeled compound is separated from the RyR-bound composition. In other embodiments of the present invention, at least 98% of the un-bound radioactively labeled compound is separated from the RyR-bound composition. In still other embodiments of the present invention, at least 95% of the un-bound radioactively labeled compound is separated from the RyR-bound composition. In yet other embodiments of the present invention, at least 90% of the un-bound radioactively labeled compound is separated from the RyR-bound composition. In yet other embodiments of the present invention, at least 75% of the un-bound radioactively labeled compound is separated from the RyR-bound composition. In yet other embodiments of the present invention, at least 50% of the un-bound radioactively labeled compound is separated from the RyR-bound composition.

In one embodiment of the present invention, step (iii) of the screen method comprises detecting and/or measuring radioactivity of the RyR-bound composition by any known manner including, but not limited to, a scintillation counter, a proportional counter, a Geiger counter, an ionization counter or any other means in the art. Alternatively, step (iii) of the screen method involves the use of a scintillation Proximity Assay (SPA). This assay uses SPA Beads which are microscopic beads which contain a scintillant that can be stimulated to emit light. This stimulation event only occurs when radiolabeled molecules of interest are bound to the surface of the bead then light is emitted that can be detected on standard scintillation counters.

In some embodiments of the present invention, whether the candidate compound has been bound to the RyR is determined based on the radioactivity of the RyR-bound composition. In certain embodiments of the present invention, the radioactivity of the RyR-bound composition is utilized to determine the amount of RyR-bound radioactively labeled compound present in the RyR-bound composition.

In certain embodiment of the present invention, the incubation conditions of step (i) are such that the radioactively labeled compound having a known affinity for the RyR would, in the absence of the candidate compound, be sufficient to completely bind the RyR. Under such conditions, one embodiment of the present invention provides for a step of determining whether the candidate compound has been bound to the RyR by comparing the amount of the RyR-bound radioactively labeled compound to the total amount of RyR incubated in step (i). In these embodiments, the difference between the amount of the RyR-bound radioactively labeled compound and the total amount of the RyR incubated in step (i) is taken to be the amount or about the amount of RyR-bound candidate compound. Thus, in these embodiments of step (iv) of the present invention, if the amount of the RyR utilized in step (i) is greater than the amount of the RyR-bound radioactively labeled compound, then the candidate material is determined to have an ability to bind (or an affinity for) the RyR.

In one embodiment of the present invention, determining whether the candidate compound has been bound to the RyR is performed by comparing the concentration or amount of radioactivity of the competitively RyR-bound composition to the concentration of radioactivity of a non-competitively RyR-bound composition. The non-competitively RyR-bound composition is prepared by incubating the same RyR with the same radioactively labeled compound in the same or similar proportion and under the same or similar conditions as utilized to prepare the competitively RyR-bound composition. In one embodiment of the present invention, the concentration of radioactivity of the non-competitively RyR-bound composition is then determined by measuring the radioactivity of the non-competitively RyR-bound composition per unit of either RyR or radioactively labeled compound. In certain embodiments of the present invention, the concentration of radioactivity of the non-competitively RyR-bound composition is then compared to the concentration of radioactivity of the competitively RyR-bound composition, wherein the radioactivity and concentration units utilized to determine the concentration of radiation of the competitively RyR-bound composition are the same as those utilized to determine the concentration of the non-

competitively RyR-bound composition. The difference between the concentration of radiation of the non-competitively RyR-bound composition and that of the competitively RyR-bound composition is or is about the concentration of the RyR-bound candidate compound. Thus, if the concentration of the radiation of the non-competitively RyR-bound composition is greater than the concentration of the radiation of the competitively RyR-bound composition, then the candidate material is determined to have an ability to bind (or an affinity for) the RyR.

In those embodiments of the present invention wherein the amount of RyR, the amount of radioactively labeled compound and the incubation conditions utilized to prepare the competitively RyR-bound composition are the same as those utilized to prepare the non-competitively RyR-bound composition, a direct comparison of the radioactivity measurements can be utilized. In such embodiments of the present invention, when the absolute radioactivity measured for the competitively RyR-bound composition is less than for the non-competitively RyR-bound composition, the presence of RyR-bound candidate compound in the competitively RyR-bound composition is affirmed.

Furthermore, the concentration of RyR-bound candidate compound and/or the concentration of RyR-bound radioactively labeled compound can also be utilized to determine the degree to which the candidate compound binds the RyR (i.e. the affinity of the candidate compound for the RyR). As the ratio of RyR-bound candidate compound to RyR-bound radioactively labeled compound increases, the affinity of the candidate compound for the RyR increases. Accordingly, in some embodiments of the present invention, the method of screening for compounds that have an ability to bind an RyR further comprises a step (v) that follows step (iv) of determining the degree to which the candidate compound binds (or has an affinity for) the RyR. The affinity of a candidate compound for an RyR can be calculated from the amount of RyR-bound candidate compound, RyR-bound radioactively labeled compound and/or the affinity known for the radioactively labeled compound utilizing methods known in the art. Similarly, the concentrations of radiation can be utilized because the concentration of the radioactivity of the RyR-bound radioactively labeled compound is proportional to the concentration of the RyR-bound radioactively labeled compound itself.

In certain embodiments of the present invention, the determination of the degree to which the candidate compound binds an RyR is determined quantitatively. In other

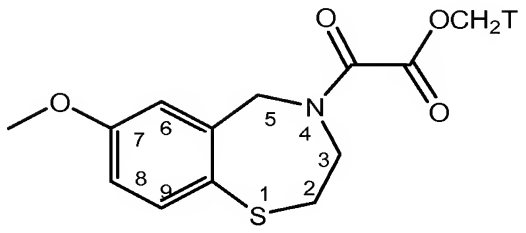
embodiments of the present invention, the determination of the degree to which the candidate compound binds an RyR is determined qualitatively. For example, in one embodiment of the present invention, wherein the degree to which the candidate compound binds an RyR is determined by comparing the radioactivity of the competitively RyR-bound composition to the radioactivity of a non-competitively RyR-bound composition, the determination is achieved, qualitatively, by simply determining whether or not the radioactivity of the competitively RyR-bound composition is, for example, (a) the same as, (b) a little less than, (c) about half of, or (d) a lot less than that of the non-competitively RyR-bound composition. In some embodiments of the present invention, these exemplary qualitative determinations of the difference of radioactivity of a competitively RyR-bound composition and a non-competitively RyR-bound composition are then utilized to determine that the affinity of the candidate compound for the RyR is (a) the same as or much less than, (b) less than, (c) about the same as, or (d) more than that of the radioactively labeled compound having a known affinity for the RyR, respectively. Alternatively, the concentration of the RyR-bound candidate composition, the concentration of the RyR-bound radioactively labeled compound, the affinity of the radioactively labeled compound for the RyR, or any combinations thereof, are utilized, in some embodiments of the present invention, to quantitatively determine the affinity of the candidate compound for the RyR utilizing techniques and methods known in the art.

In one embodiments of the present invention, a radioactively labeled 1,4-benzothiazepine having a known affinity for an RyR is a radioactively labeled compound of formula I comprises at least one radioactive atom and is represented by formulae I or II herein .

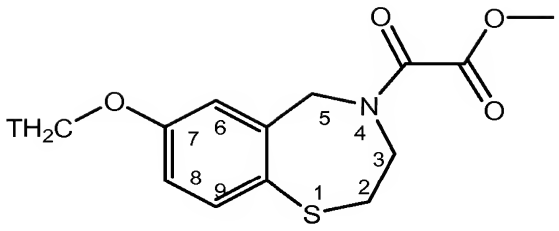
In some embodiments of the present invention, the radioactively labeled 1,4-benzothiazepine of Formula I and II having a known affinity for an RyR is radioactively labeled with any radioactive label known in the art. In specific embodiments of the present invention, the radioactive label is selected from, by way of non-limiting example, tritium (^{3}H or T), carbon-14 (^{14}C), radioactive phosphorus isotopes, such as phosphorus-32 (^{32}P) and phosphorus-33 (^{33}P), sulfur-35 (^{35}S), and radioactive halogens, which include, but are not limited to, chlorine-36 (^{36}Cl), iodine-123 (^{123}I), iodine-125 (^{125}I), iodine-126 (^{126}I), iodine-129 (^{129}I) and iodine-131 (^{131}I). In one embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine having a known affinity for an RyR comprises a compound of

formula I and II wherein one or more of the hydrogen (^{1}H) atoms is replaced with a tritium (^{3}H) atom. In another embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine having a known affinity for an RyR comprises a compound of formula I and II wherein the sulfur atom at the 1-position of the 1,4-benzothiazepine is a sulfur-35 (^{35}S) atom. In another embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine having a known affinity for an RyR comprises a compound of formula II wherein one or more carbon atoms on the 1,4-benzothiazepine ring or on an appended group atom is replaced by a ^{14}C or ^{11}C atom.

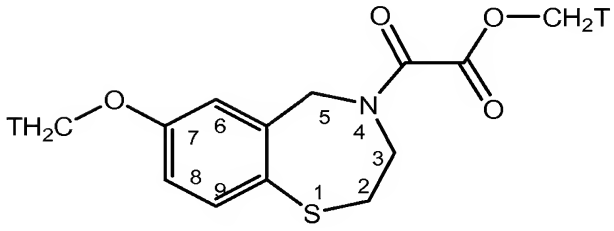
In specific embodiments of the present invention, the radioactively labeled 1,4-benzothiazepine having a known affinity for an RyR is **$^{3}H_1$-Compound 1-a**. In another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **$^{3}H_1$-Compound 1-b**. In still another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **$^{3}H_2$-Compound 1**. In yet another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **^{3}H-Compound 2**. In yet another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **^{3}H-Compound 2a**. In yet another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **^{3}H-Compound 3**. In yet another specific embodiment of the present invention, the radioactively labeled 1,4-benzothiazepine compound having a known affinity for an RyR is **^{14}C-Compound 3**.



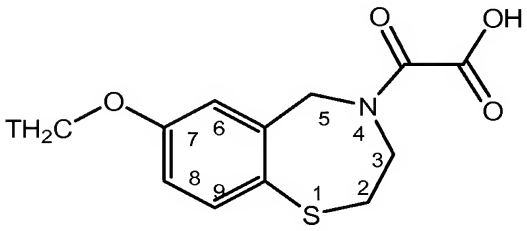
$^{3}H_1$-Compound 1-a



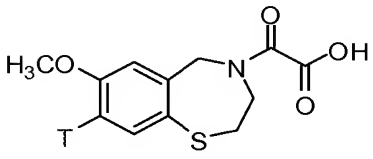
$^{3}H_{1}$-Compound 1-b



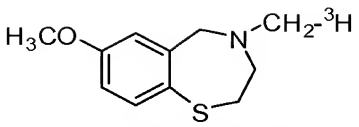
$^{3}H_{2}$-Compound 1



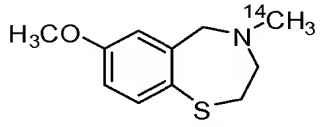
^{3}H-Compound 2



^{3}H-Compound 2a



^{3}H- **Compound 3**



^{14}C- **Compound 3**

It will be apparent to a person of skill in the art that more than one hydrogen on the methyl at the 7-position and/or the methyl on the oxalic acid moiety can be substituted by tritium (^{3}H or T). Thus, the present invention contemplates any one of compounds $^{3}H_1$-Compound 1-a, $^{3}H_1$-Compound 1-b, $^{3}H_2$-Compound 1, ^{3}H-Compound 2 or ^{3}H-Compound 3 wherein one or more CH_2T groups is replaced with CT_2H or CT_3, or any combination thereof.

Because methods of certain embodiments of the present invention are useful for identifying compounds with high affinity for an RyR and RyRs are associated with various disorders and diseases, methods of the present invention are useful for determining compounds useful in the treatment of disorders and diseases associated with RyRs. Disorders and diseases associated with the RyRs include, but are not limited to, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; CPVT; and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss.

In one embodiment of the present invention, the invention provides a method for treating a disease or disorder associated with an RyR by administering to a subject a compound identified as having an affinity for the RyR.

In some embodiments of the present invention, the compounds identified as having an ability to bind RyR are identified via a method of screening for candidate compounds that bind an RyR comprising the steps of: (i) incubating the RyR with a radioactively labeled compound in the presence or absence of a candidate compound so as to prepare a competitively bound-RyR composition containing RyR-bound candidate compound, RyR-bound radioactively labeled compound, or a combination thereof; (ii) separating the RyR-bound composition from un-bound radioactively labeled compound; (iii) measuring the radioactivity of the competitively RyR-bound composition; and (iv) determining whether the candidate compound binds the RyR based on the proportion of RyR-bound radioactively labeled compound in the presence and absence of the candidate compound.

In yet another embodiment of the present invention, the invention provides for a method of measuring tissue distribution of a radioactively labeled compound, which method comprises administering the radioactively labeled compound to a subject and measuring the amount of the radioactively labeled compound in the subject.

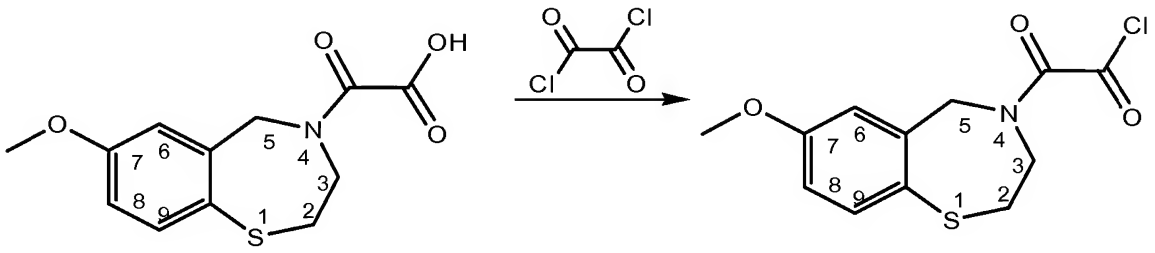
In yet another embodiment of the present invention, the invention provides for a method of measuring the tissue distribution of a radioactively labeled compound, which method comprises isolating a tissue from a subject, contacting the tissue with the radioactively labeled compound and measuring the amount of the radioactively labeled compound in the tissue.

The following examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

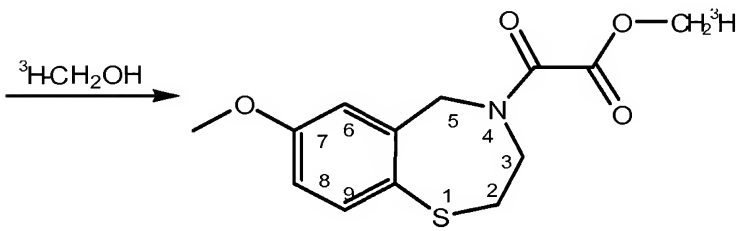
EXAMPLES

Example 1: Synthesis of ^{3}H-Compound 1-a:

(1) Synthesis method A of ^{3}H-Compound 1-a: The radioactively labeled Compound ^{3}H-Compound 1-a is prepared according to the process set forth below. The process is illustrated in Scheme 1.



Compound 2



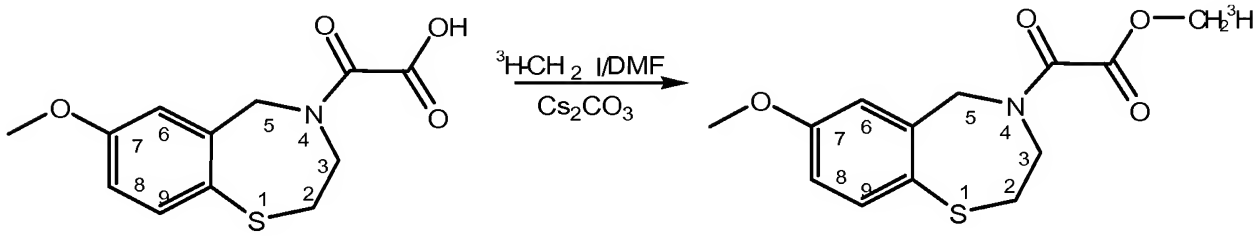
^{3}H-Compound 1-a

Scheme 1

Oxalyl chloride (2.0 M solution in CH_2Cl_2, 0.1 ml, 0.2 mmol, excess, Aldrich) was added to Compound 2 (5 mg, 0.019 mmol) in anhydrous CH_2Cl_2 (5 ml). The reaction mixture was stirred at room temperature for 1 hour. The solvents were removed at 30° C under reduced pressure (rotary evaporator). The residue was dissolved in CH_2Cl_2 (3 ml) and treated with a ^{3}H-MeOH solution in CH_2Cl_2 (0.73 mg in 0.1 ml CH_2Cl_2, 0.023 mmol, 1.2 eq.) and (i-$C_3H_7)_2$NEt solution in CH_2Cl_2 (3 mg in 0.1 ml CH_2Cl_2). The reaction mixture was stirred for 10 min and washed with 1N HCl (1X 2ml), and a sat. $NaHCO_3$ solution (3X3 ml). The solvents were removed under reduced pressure. TLC and NMR showed that the crude mixture contains ^{3}H-Compound 1-a, which was further purified by SiO_2 chromatography (CH_2Cl_2). Yield: 85%. Structure of the title compound was confirmed by TLC and NMR and by comparison with the non-radioactively labeled analog of ^{3}H-Compound 1-a prepared

by other methods. It is noted that more than one hydrogen in the compound of formula 2 can be substituted by tritium, i.e., the methyl position can be substituted by one, two or three tritium atoms.

(2) Synthesis method B of ^{3}H-Compound 1-a: The Tritium labeled Compound 1-a was prepared according to the process set forth below. The process is illustrated in Scheme 2.

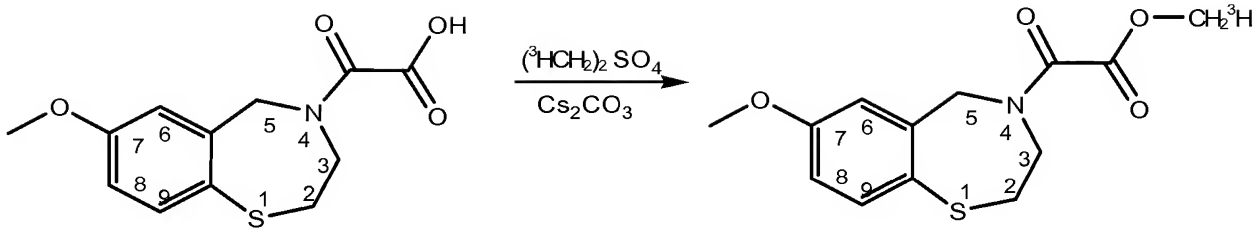


Compound 2 ^{3}H-Compound 1-a

Scheme 2

To Compound 2 (0.6 mg, 0.0022 mmol) in anhydrous DMF (0.1 ml) was added tritiated methyl iodide in DMF (freshly prepared, 0.4 mg in 0.05 ml DMF, 0.0023 mmol, 1.3 eq.) and Cs_2CO_3 (2 mg, 0.006 mmol). The reaction mixture was stirred at room temperature for 8 hours. The solvent (DMF) was removed by blowing with Argon. The residue was dissolved in CH_2Cl_2 (2 ml) and TLC showed ^{3}H-Compound 1-a is the major product compared with standard non-radioactively labeled analog of ^{3}H-Compound 1-a. The yield was estimated to be ~60-70% based on the yield from larger scale preparation (5 mg scale) and the structure of the product was confirmed by NMR and MS on larger scale preparation (5 mg scale). It is noted that more than one hydrogen in the compound of formula 2 can be substituted by tritium, i.e., the methyl position can be substituted by one, two or three tritium atoms.

(3) Synthesis method C of ^{3}H-Compound 1-a: The Tritium labeled Compound ^{3}H-Compound 1-a was prepared according to the process set forth below. The process is illustrated in Scheme 3.



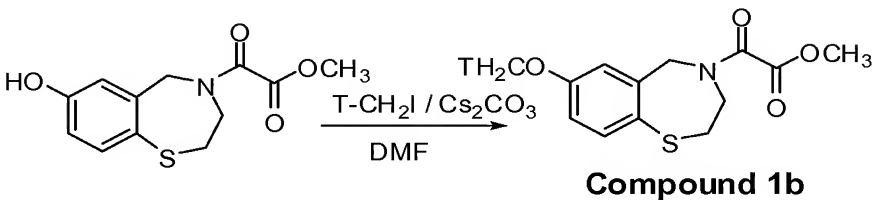
Compound 2 ^{3}H-Compound 1-a

Scheme 3

^{3}H-Compound 1-a was prepared according to Scheme 3 in a manner similar to Scheme 2 in ~60% yield. More than one hydrogen in the compound ^{3}H-Compound 1-a can be substituted by tritium.

Example 2: Synthesis of ^{3}H-Compound 1-b:

Synthesis method of ^{3}H-Compound 1-b: The radioactively labeled Compound ^{3}H-Compound 1-b was prepared according to the process set forth below. The process is illustrated in Scheme 4.

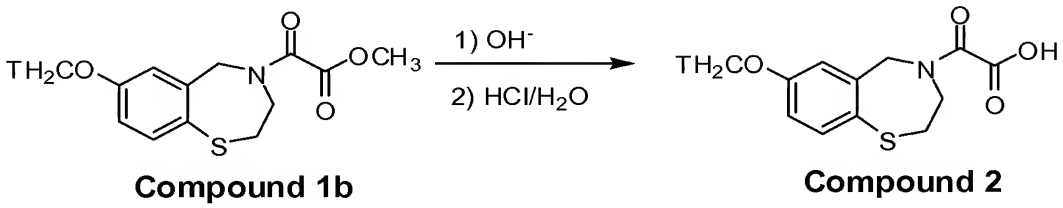


Scheme 4

To the phenol precursor (0.55 mg, 0.0020 mmol) in anhydrous DMF (0.1 ml) was added tritiated methyl iodide in DMF (freshly prepared, 0.4 mg in 0.05 ml DMF, 0.0023 mmol, 1.3 eq.) and Cs_2CO_3 (2 mg, 0.006 mmol). The reaction mixture was stirred at room temperature for 12 hours. The solvent (DMF) is removed by blowing with argon to provide crude ^{3}H-Compound 1-b in yield of ~70% (the structure and the yield are based on larger scale preparation: 10 mg scale). More than one hydrogen in the compound of formula 2 can be substituted by tritium.

Example 3: Synthesis of ^{3}H-Compound 2:

Synthesis method of ^{3}H-Compound 2: The radioactively labeled Compound **^{3}H-Compound 2** was prepared according to the process set forth below. The process is illustrated in Scheme 5.

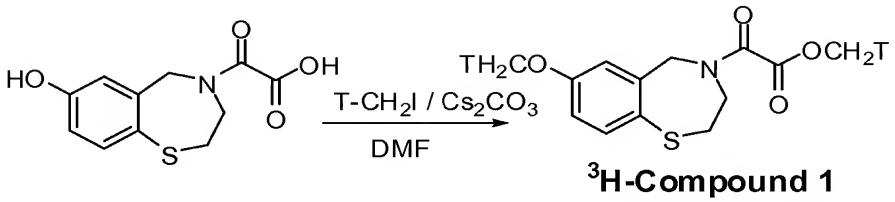


Scheme 5

The crude **^{3}H-Compound 1-b** prepared according to Example 2 was dissolved in 0.3 ml of methanol solution containing 0.05 ml 1N NaOH and the reaction mixture was stirred for 8 hours. TLC showed no **^{3}H-Compound 1-b** in the reaction mixture. The solvents were removed by blowing with argon and the residue was dissolved in 1 ml of H_2O. The aqueous phase was washed with ethyl acetate (1 ml, discarded) and acidified to a pH of about 3-4 with 1N HCl. The product **^{3}H-Compound 2** was extracted with ethyl acetate (3X1ml). Evaporation of the organic phase provides **^{3}H-Compound 2** (confirmed by TLC, CH_2Cl_2:MeOH=5:1 and MS). The yield is estimated to be > 90% and purity is estimated be >90% based on larger scale preparations. More than one hydrogen in the compound of formula 2 can be substituted by tritium.

Example 4: Synthesis of $^3H_2$-Compound 1:

The radioactively labeled Compound **$^3H_2$-Compound 1** was prepared according to the process set forth below. The process is illustrated in Scheme 6.

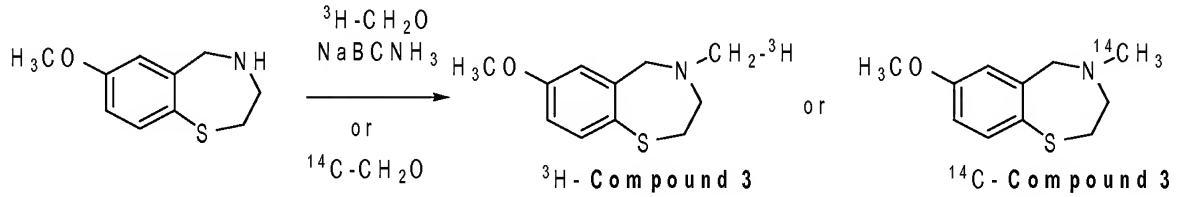


Scheme 6

To the phenol precursor (0.55 mg, 0.0020 mmol) in anhydrous DMF (0.1 ml) was added tritiated methyl iodide in DMF (freshly prepared, 0.8 mg in 0.1 ml DMF, 0.0046 mmol, 2.6 eq.) and Cs_2CO_3 (2 mg, 0.006 mmol). The reaction mixture is stirred at room temperature for 12 hours. The solvent (DMF) is removed by blowing with argon to provide crude **^{3}H-Compound 1** in yield of ~70% (the structure and the yield are based on larger scale preparation: 10 mg scale). It is noted that more than one hydrogen in the compound of formula 1 can be substituted by tritium.

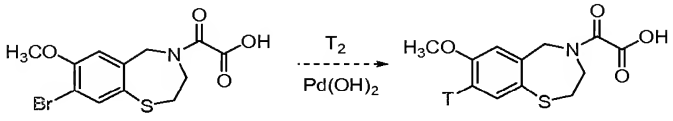
Example 5: Synthesis of radiolabeled Compound 3

Synthesis method of ^{3}H-Compound 3 or ^{14}C-Compound 3: The radioactively labeled Compound **^{3}H-Compound 3 or ^{14}C-Compound 3** is prepared according to the process set forth below.



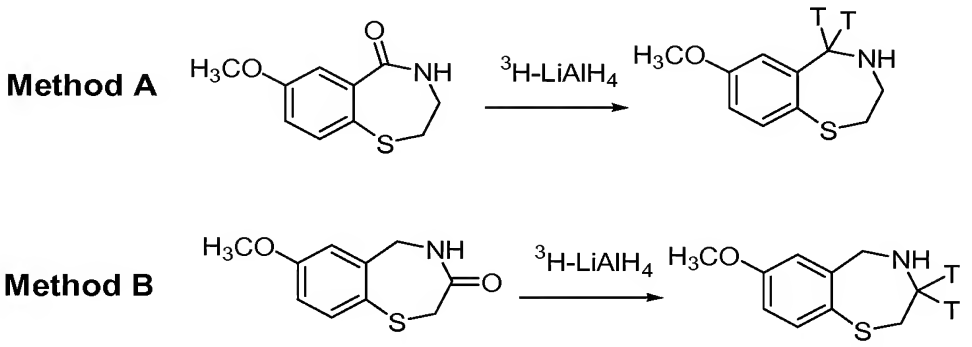
Example 6 - Transformation of Br-analogs to H-analogs:

Synthesis method of ^{3}H-Compound 2a: The radioactively labeled Compound **^{3}H-Compound 2a** can be prepared according to the process set forth below.



5-Br-Compound-2a ^{3}H-Compound-2a

Example 7: Synthesis of ^{3}H-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine



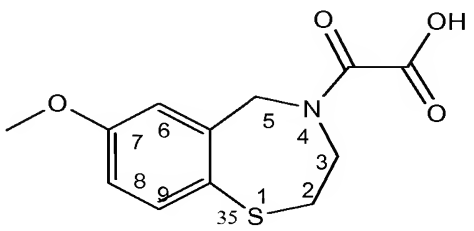
Method A: Synthesis of 5,5-di-^{3}H-benzothiazepine: 7-methoxy-5-oxo-2,3,4,5-tetrahydro-1,4-benzothiazepine(0.047 mg) was treated with ^{3}H-LiAlH$_4$ (1 eq) in THF (5 ml) at 60C overnight. After being cooled to room temperature, $Na_2SO_4.10H_2O$ (5 mg) was added slowly. The resulting mixture was stirred at room temperature for one hour and filtered through a short celite column. Removal of the solvents gave the desired product, ^{3}H-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine, (yield: 90%).

Method B: Synthesis of 3,3-di-^{3}H-benzothiazepine: 7-methoxy-3-oxo-2,3,4,5-tetrahydro-1,4-benzothiazepine(0.047 mg) was treated with 3H-LiAlH$_4$ (1 eq) in THF (5 ml) at 60C overnight. After being cooled to room temperature, $Na_2SO_4.10H_2O$ (5 mg) was added slowly. The resulting mixture was stirred at room temperature for one hour and filtered through a short celite column. Removal of the solvents gave the desired product, ^{3}H-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine.

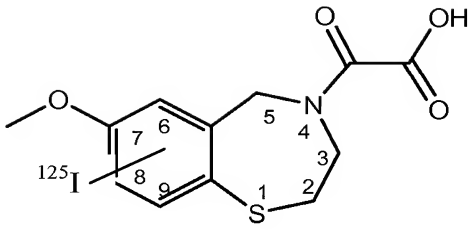
Example 8- Radioactively labeled Compound 2

Additional radioactive derivatives of compound 2 are set forth below.

^{35}S-Compound 2



^{125}I-Compound 2



Example 9 - Assay screening for compounds capable of binding an RyR:

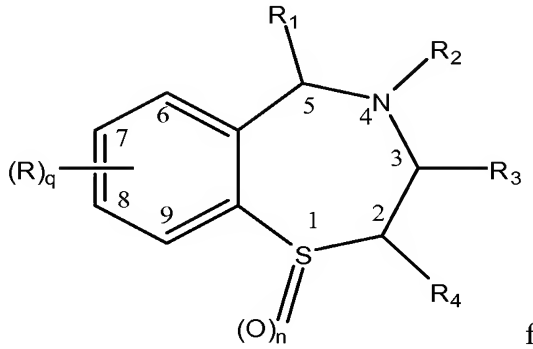
The assay for screening for compounds capable of binding an RyR is based on the ability of test compounds to compete with a radioactively labeled compound of known affinity for the RyR. In this assay, the RyR is incubated with a mixture of radioactively labeled compound and candidate compounds of known concentrations for 1 hour at room temperature. After incubation, the reaction mixture is filtered through Whatman GF/B filters and washed with ice cold buffer (170 mM KCL, 10 mM MOPS). The amount of radioactivity retained on the filter (i.e., associated with the RyR) is determined by scintillation counts. Candidate compounds that bind to RyR and compete with the radioactively labeled compound decrease the measured cpms on the filter.

While certain embodiments of the invention have been illustrated and described, it will be clear that the invention is not limited to the embodiments described herein. Numerous modifications, changes, variations, substitutions and equivalents will be apparent to those skilled in the art without departing from the spirit and scope of the present invention as described by the claims, which follow.

THE CLAIMS

What is claimed is:

1. A radioactively labeled compound, represented by Formula I:



formula I

wherein:

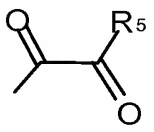
n is selected from the group consisting of 0, 1 and 2;

q is selected from the group consisting of 0, 1, 2, 3, and 4;

each R is independently selected from the group consisting of halogen, R_4, $-OR_4$; $-N(R_4)_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3R_4$, $-S(=O)_2R_4$, $-S(=O)R_4$, $OS(=O)_2CF_3$, acyl, -O-acyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, alkylaryl, heterocyclylalkyl, alkynyl, (hetero-)arylthio, and (hetero-) arylamino;

R_1 is selected from the group consisting of oxo (=O) and R_4;

R_2 is selected from the group consisting of alkyl, alkenyl, alkynyl and



R_3 is selected from the group consisting of oxo (=O), R_4, $-CO_2R_4$, $-C(=O)N(R_4)_2$, acyl, and -O-acyl;

each R_4 is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; and

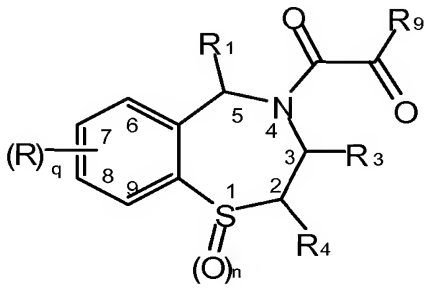
R_9 is selected from the group consisting of halogen, R_4, $-OR_4$, $-N(R_4)_2$, $-N(R_4)_2N(R_4)_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3R_4$, $-S(=O)_2R_4$, $-S(=O)R_4$, $-OS(=O)_2CF_3$, acyl, -O-acyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, heterocyclylalkyl, alkynyl, (hetero-)arylthio, and (hetero-) arylamino;

wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)arylthio, and (hetero-)arylamino is unsubstituted or substituted with one or more groups selected from the group consisting of halogen, CF_3, OCF_3, cyano, nitro, N_3, oxo, alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, aryl, alkylaryl, heteroaryl, OR_a, SR_a, $S(=O)R_e$, $S(=O)_2R_e$, $P(=O)_2R_e$, $S(=O)_2OR_a$, $P(=O)_2OR_a$, NR_bR_c, $NR_bS(=O)_2R_e$, $NR_bP(=O)_2R_e$, $S(=O)_2NR_bR_c$, $P(=O)_2NR_bR_c$, $C(=O)OR_a$, $C(=O)R_a$, $C(=O)NR_bR_c$, $OC(=O)R_a$, $OC(=O)NR_bR_c$, $NR_bC(=O)OR_a$, $NR_dC(=O)NR_bR_c$, $NR_dS(=O)_2NR_bR_c$, $NR_dP(=O)_2NR_bR_c$, $NR_bC(=O)R_a$, or $NR_bP(=O)_2R_e$, wherein R_a, R_b, R_c, R_d and R_e are each independently R_4; or said R_b and R_c together with the N to which they are bonded optionally form a heterocyclyl or heteroaryl;

wherein at least one atom in the compound of formula I is radioactively labeled; and

all enantiomers, diastereomers, tautomers, salts, hydrates, solvates, complexes, and prodrugs of the compounds of Formula I.

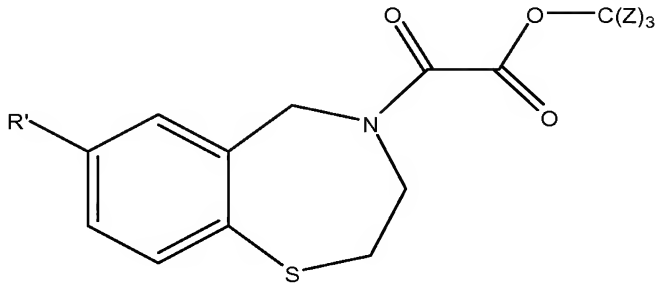
2. A radioactively labeled compound represented by formula II



formula II

wherein each of n, q, R, R_1, R_3, R_4 and R_9 are as defined in claim 1.

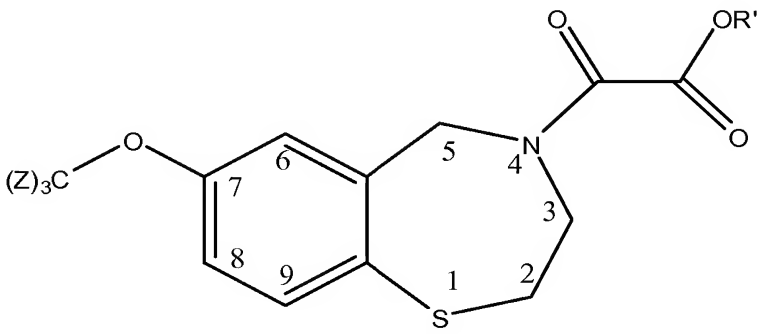
3. A radioactively labeled compound of claim 2, represented by the formula:



wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F.

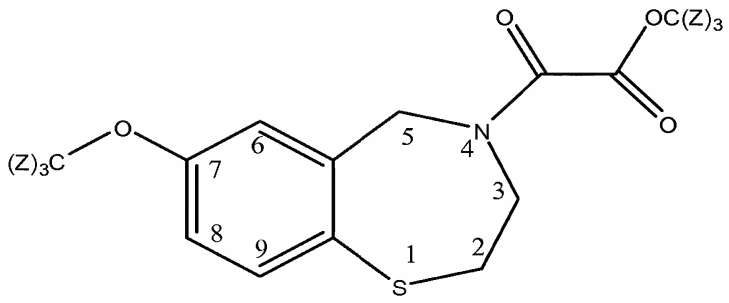
4. A radioactively labeled compound of claim 3, wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen or ^{3}H, with at least one Z being ^{3}H.

5. A radioactively labeled compound of claim 2, represented by the formula:



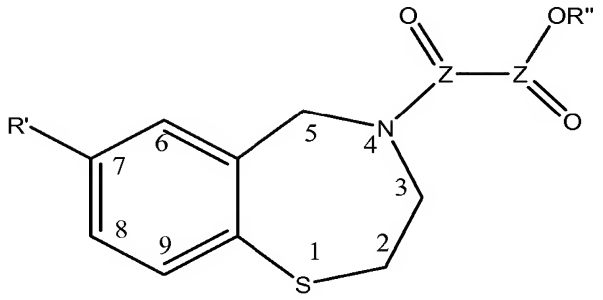
wherein R' is hydrogen or methyl and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F.

6. A radioactively labeled compound of claim 2, represented by the formula:



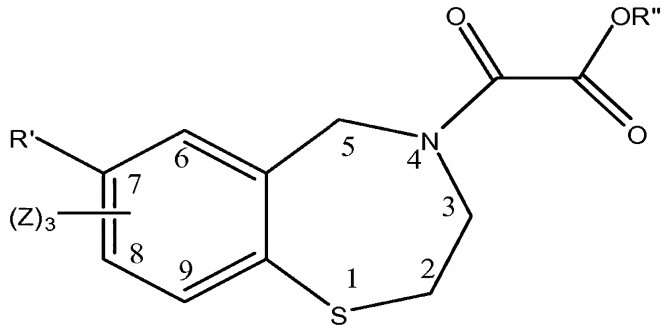
wherein each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F.

7. A radioactively labeled compound of claim 2, represented by the formula:



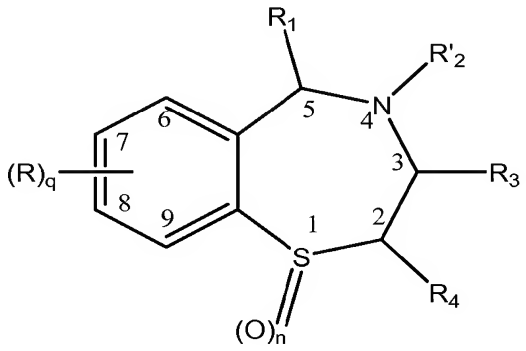
wherein R' is hydrogen or methoxy, R'' is hydrogen or methyl, and each Z, independently of the other, is C, ^{11}C or ^{14}C, with at least one Z being ^{11}C or ^{14}C,.

8. A radioactively labeled compound of claim 2, represented by the formula:



wherein R' is hydrogen or methoxy, R'' is hydrogen or methyl, and each Z, independently of the other, is hydrogen, ^{3}H, ^{18}F or ^{125}I, with at least one Z being ^{3}H, ^{18}F or ^{125}I.

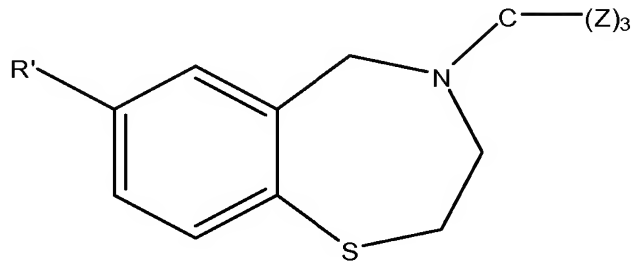
9. A radioactively labeled compound represented by formula III:



formula III

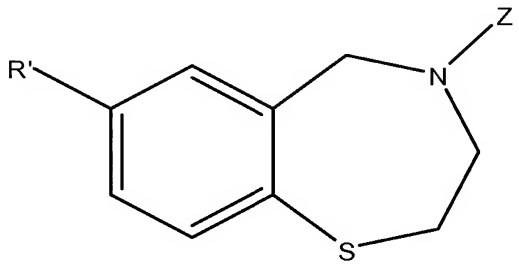
wherein R'_2 is alkyl, alkenyl or alkynyl, and wherein each of n, q, R, R_1, R_3 and R_4 are as defined in claim 1.

10. A radioactively labeled compound of claim 9, represented by the formula:



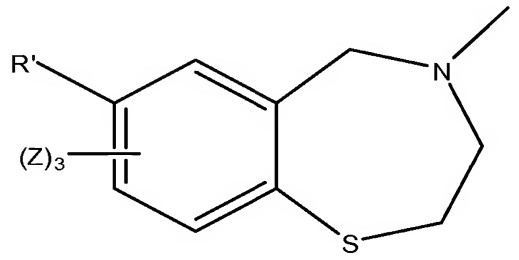
wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at one Z being ^{3}H or ^{18}F.

11. A radioactively labeled compound of claim 9, represented by the formula:



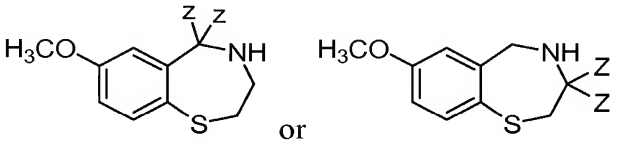
wherein Z is $^{11}CH_3$ or $^{14}CH_3$, and R' is hydrogen or methoxy.

12. A radioactively labeled compound of claim 9, represented by the formula:



wherein R' is hydrogen or methoxy and each Z, independently of the other, is hydrogen, ^{3}H, ^{18}F, or ^{125}I, with at least one Z being ^{3}H, ^{18}F, or ^{125}I.

13. A radioactively labeled compound represented by the structure



wherein each Z is H or ^{3}H, wherein at least one Z is ^{3}H.

14. The radioactively labeled compound of claim 1 wherein the radioactive atom is selected from the group consisting of tritium (^{3}H or T), carbon-14 (^{14}C), carbon-11 (^{11}C), radioactive phosphorus isotopes, sulfur-35 (^{35}S), and radioactive halogen.

15. The radioactively labeled compound of claim 14 wherein the radioactive atom is sulfur-35 (^{35}S) at the 1-position of the 1,4-benzothiazepine ring.

16. The radioactively labeled compound of claim 14 wherein the radioactive atom is ^{3}H.

17. The radioactively labeled compound of claim 14 wherein the radioactively labeled atom is carbon-14 (^{14}C) and is present as a ring carbon or pendant carbon.

18. The radioactively labeled compound of claim 14 wherein the radioactively labeled atom is a radioactive halogen.

19. The radioactively labeled compound of claim 18 wherein the radioactive halogen is selected from the group consisting of chlorine-36 (^{36}Cl), fluorine-18 (^{18}F) iodine-123 (^{123}I), iodine-125 (^{125}I), iodine-126 (^{126}I), iodine-129 (^{129}I) and iodine-131 (^{131}I).

20. The radioactively labeled compound of claim 19 wherein the radioactive halogen is iodine-125 (^{125}I) or fluorine-18 (^{18}F).

21. A method of screening for candidate compounds that bind a ryanodine receptor (RyR) comprising the steps of:

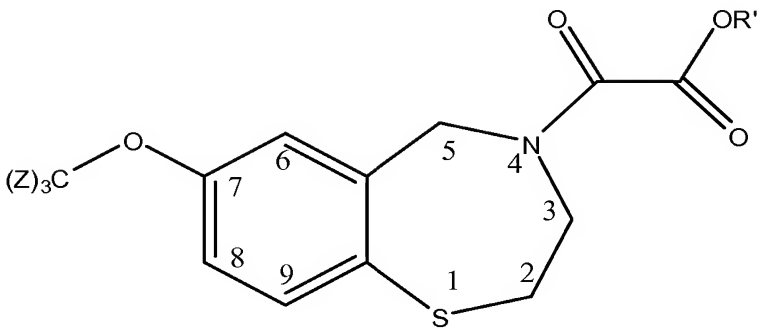
incubating the RyR with a radioactively labeled compound according to claim 1 in the presence or absence of a non-radioactive candidate compound so as to prepare a competitively bound-RyR composition containing RyR-bound candidate compound, RyR-bound radioactively labeled compound, or a combination thereof;

separating the RyR-bound composition from un-bound radioactively labeled compound;

measuring the radioactivity of the competitively RyR-bound composition; and

determining whether the candidate compound binds the RyR based on the proportion of RyR-bound radioactively labeled compound in the presence and absence of the candidate compound.

22. The method of claim 21, wherein the radioactively labeled compound is represented by the structure:



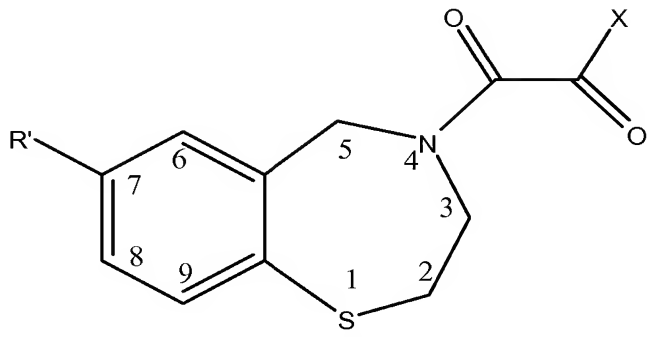
wherein R' is hydrogen or methyl and each Z, independently of the other, is hydrogen, ^{3}H or ^{18}F, with at least one Z being ^{3}H or ^{18}F.

23. The method of claim 21, which is adapted for high-throughput screening of a plurality of candidate compounds.

24. The method of claim 21, wherein the RyR is selected from the group consisting of RyR1, RyR2 and RyR3.

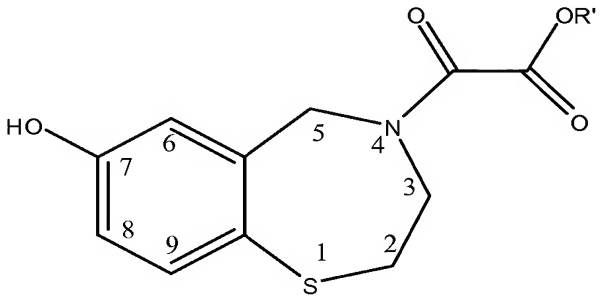
25. A method of treating a disease or disorder associated with an RyR by administering to a patient in need thereof a therapeutically active compound having the ability to bind the RyR, wherein the compound is identified according to the method of claim 21.

26. A method of preparing a compound according to claim 3 which comprises preparing an intermediate compound having the structure



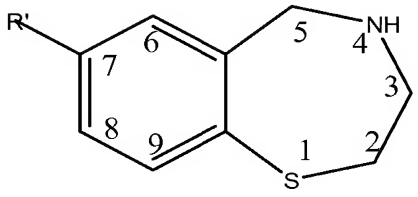
wherein X is a leaving group, and treating the intermediate compound with $(Z)_3COH$ under conditions sufficient to substitute a $(Z)_3CO$ moiety for the X moiety of the intermediate compound to form a radiolabeled compound.

27. A method of preparing a compound according to claim 5 which comprises preparing an intermediate compound having the structure

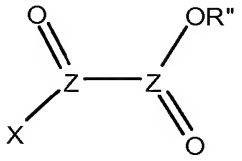


and treating the intermediate compound with $(Z)_3CX$ wherein X is a leaving group under conditions sufficient to convert the hydroxyl moiety of the intermediate compound into a $(Z)_3CO$ moiety to form a radiolabeled compound.

28. A method of preparing a compound according to claim 7 which comprises preparing an intermediate compound having the formula

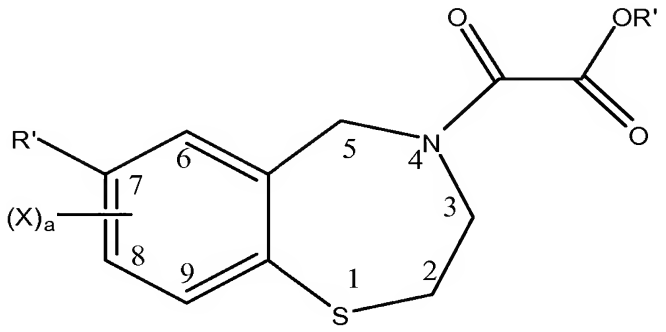


and reacting the intermediate compound with a group of the formula:



wherein X is a leaving group under conditions sufficient to form a radiolabeled compound.

29. A method of preparing a compound according to claim 8 which comprises preparing an intermediate compound having the formula



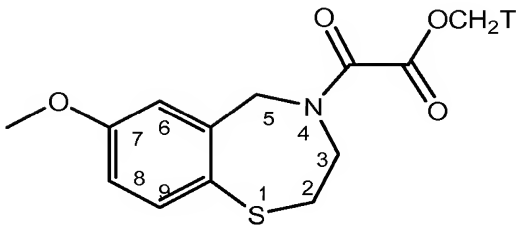
wherein X is a leaving group and a is an integer selected from 1, 2 and 3; and substituting at least one X moiety with Z so as to form a radiolabeled compound.

30. A method of measuring tissue distribution of a radioactively labeled compound according to claim 1, which method comprises administering the radioactively labeled compound to a subject and measuring the amount of the radioactively labeled compound in the subject.

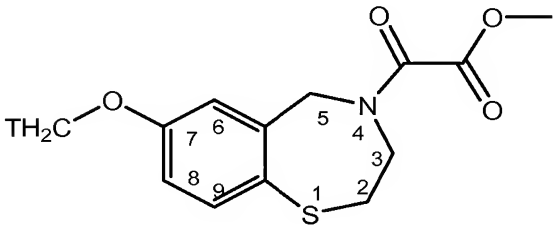
31. A method of measuring the tissue distribution of a radioactively labeled compound according to claim 1, which method comprises isolating a tissue from a subject, contacting the tissue with the radioactively labeled compound and measuring the amount of the radioactively labeled compound in the tissue.

32. Use of a radioactively labeled compound according to claim 1 in the preparation of a pharmaceutical composition that can be administered for determining binding to ryanodine receptors or for measuring tissue distribution in a subject.

33. A compound selected from the group consisting of:



;



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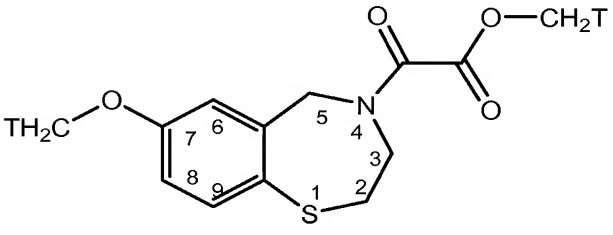

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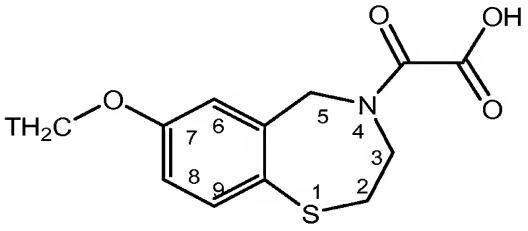
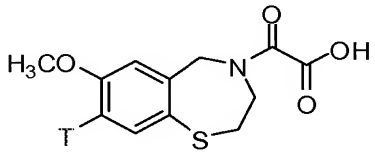
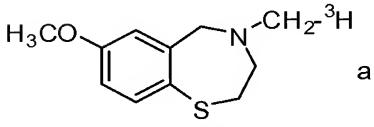


5 ;

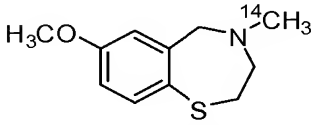


;



and

^{3}H- Compound 3



^{14}C- Compound 3